Washington Department of Fish and Wildlife

2007-2009 Biennial Report:

Invasive Species Tunicate Response in the Puget Sound Region

Prepared for the
Tunicate Response Advisory Committee and
The Puget Sound Partnership

by

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Introduction

There are seven non-native tunicates currently reported as established to some degree in Washington State waters (Table 1). Three of these are of primary invasive concern to the Washington Department of Fish and Wildlife (WDFW) resource managers and local stakeholders and are the focus of the WDFW Tunicate Management Plan and this report. The remaining four are of secondary invasive concern as they have not demonstrated a high invasive threat.

Table 1. Known nonnative invasive tunicates in Washington State marine waters and their management priority.

Scientific Name	Common Name	Priority Level
Styela clava	Solitary Club Tunicate	High
Ciona savignyi	Transparent Solitary Tunicate	High
Didemnum vexillum	Colonial Tunicate	High
Botryllus schlosseri	Colonial Star Tunicate	Low
Botrylloides violaceus	Colonial Sheath Tunicate	Low
Ciona intestinalis	Solitary Tunicate	Low
Molgula manhattensis	Solitary Sea Grape Tunicate	Low

The Puget Sound Partnership (PSP) has contracted with the WDFW Aquatic Invasive Species Unit (AISU) to provide a continued response to the threat of non-native tunicates in Puget Sound for the 2007-2009 biennium (Interagency Contract #200804). The work is to be conducted through consultation with the state Tunicate Response Caucus and the Tunicate Response Advisory Committee (TRAC). The agreement implements or addresses:

a) Unfinished elements of the 2006-2007 Interagency Invasive Species Rapid Response Plan.

b) The Aquatic Nuisance Species (ANS) Management Plan by minimizing the introduction of aquatic nuisance species; stopping them from spreading; and eradicating or controlling them to minimize impacts.

c) The Puget Sound Recovery Plan by protecting and preventing loss of habitat; restoring habitat functions and values; protecting ecosystem biodiversity; and building and sustaining capacity for action.

d) The WDFW Tunicate Management Plan.

Further, there are seven specific expected outcomes and results listed in the contract as follows:

1) Develop a statewide tunicate management plan that includes a priority system for managing invasive tunicates to include eradication objectives maximize the use of limited resources, and that identifies long-term strategies.

2) Implement a research and monitoring program for non-native tunicates.

3) Assist in the development of best management practice manuals for managing invasive marine organisms at sites such as marinas, boat yards, aquaculture operations, and hull cleaning services; and define clear strategies to implement these practices.

4) Survey a minimum of 50 high risk areas such as marinas, boat cleaning areas, and shellfish growing areas for the presence or absence of invasive tunicates.

5) Map locations of infestations, and make this information available to the legislature, resource agencies, and the public.

6) Post all management plans, best management practices manuals, management methods, and reports on the WDFW web page for public access.

7) Prepare cost estimates by June 30, 2009 of additional work needed to successfully eradicate invasive tunicates within Puget Sound.

This report details AISU efforts toward meeting the goals and objectives of the contract and makes recommendations for further work into the 2009-2011 biennium.

Summary

Ten tunicate management regions were defined for Washington's marine waters. One hundred sites have been surveyed for the presence of non-native tunicates. Thirty one sites across seven management regions were found to have one or more of the three non-native tunicates of greatest management concern.

Five *Didemnum vexillum* eradication methods were tested and evaluated at Dockton Park in central Puget Sound. Three of the treatments proved to be highly effective at killing all or most of the *D. vexillum* present, one method was partially effective, and one method had little or no effect. Subsequent to the treatment trials, all *D. vexillum* was removed from the facility.

Thorough presence/absence macrofauna community structure surveys were conducted at four marinas known to be infested with one or more species of non-native tunicates. Robust estimates of non-native tunicate densities were produced for each of the four facilities. Additionally, a community structure survey was conducted at a marina considered to be at high risk of infestation but that is not presently known to be infested.

Non-native tunicate removals from vessel hulls were conducted annually at six marinas known to be infested with one or more of the three non-native tunicate species of greatest managent

concern. Some vessels from which non-native tunicates had been removed during initial efforts were found to be reinfested during subsequent removals.

A rapid response team was dispatched to inspect a large vessel that had been recently towed to Puget Sound from China. The vessel had been sitting idle in a harbor known to have tunicates not native to the Pacific Northwest. No non-native tunicates were found.

A qualitative survey of *Ciona savignyi* along a deep-water rock wall in southern Hood Canal was conducted using a surface tethered remotely operated vehicle. *Ciona savignyi* was found to be abundant and nearly uniformly distributed along the full length and depth of the wall. The ROV proved to be a valuable tool for deep-water observations and showed promise for collecting quantitative assessment data on tunicate distribution and abundance in deep-water habitat.

Non-Native Tunicate Management Regions and Mapped Compilation of Surveyed Sites

In order to facilitate rapid response preparedness, the marine waters of Washington State were divided into ten management regions. A variety of factors were taken into consideration for the placement of the regional boundary lines including physical hydrology, bio-regimes, political jurisdictions, and the potential placement of regional or multiregional rapid response staging units (Plate 1).

Thirty one of 100 sites surveyed among seven management regions contained one or more of the three priority species of non-native tunicate (*Styela clava*, *D. vexillum*, *C. savignyi*). While *S. clava* appears to be somewhat restricted in its distribution, the occurrence of *D. vexillum* and *C. savignyi* is relatively widespread at isolated locations throughout greater Puget Sound (Plate 2). Locations for each of the 100 sites surveyed are designated on Plates 3 and 4.

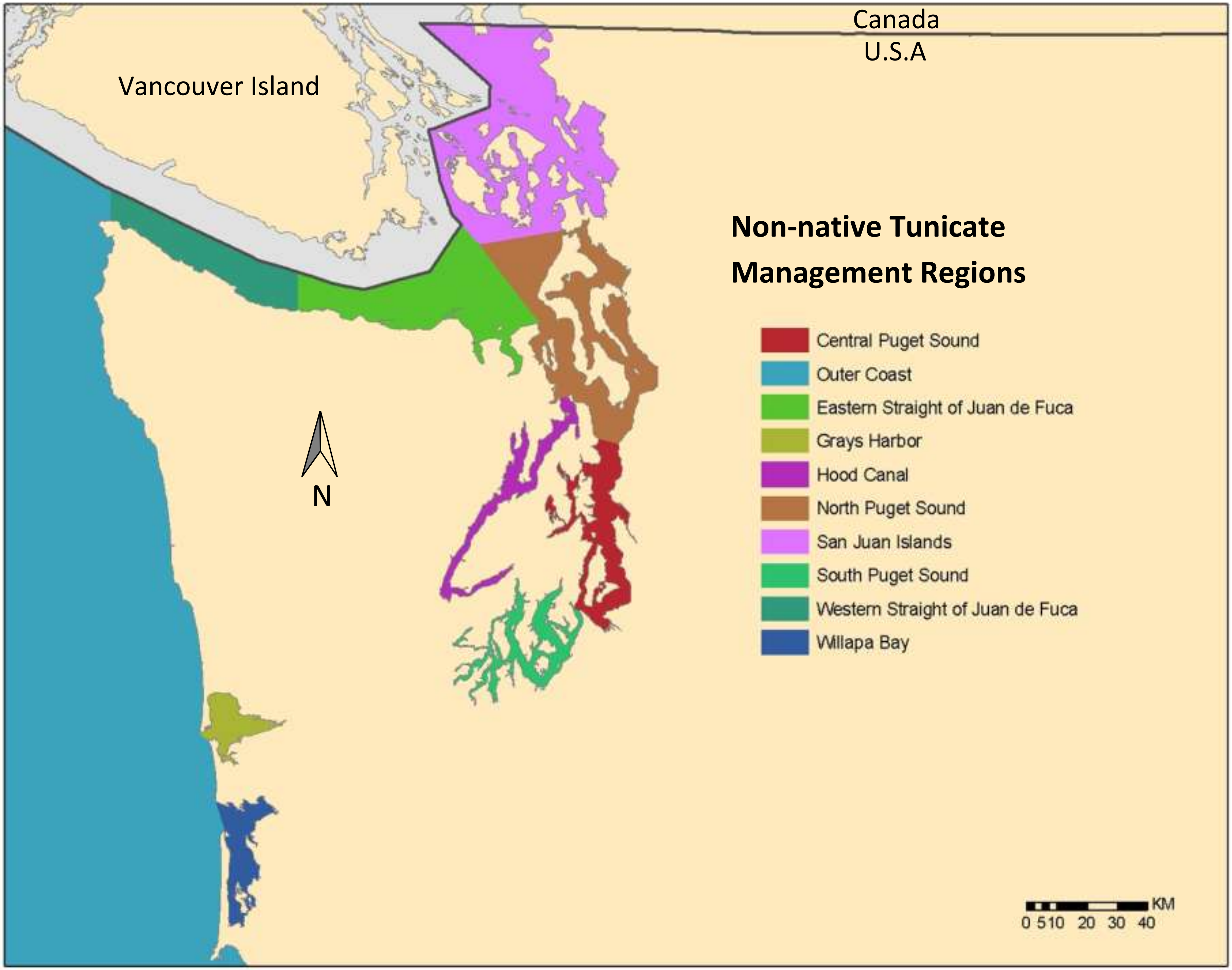

Canada
U.S.A
Vancouver Island
Non-native Tunicate
Management Regions
Central Puget Sound
Outer Coast
Eastern Straight of Juan de Fuca
Grays Harbor
Hood Canal
North Puget Sound
San Juan Islands
South Puget Sound
Western Straight of Juan de Fuca
Willapa Bay
N
KM
0 5 10 20 30 40

Plate 1

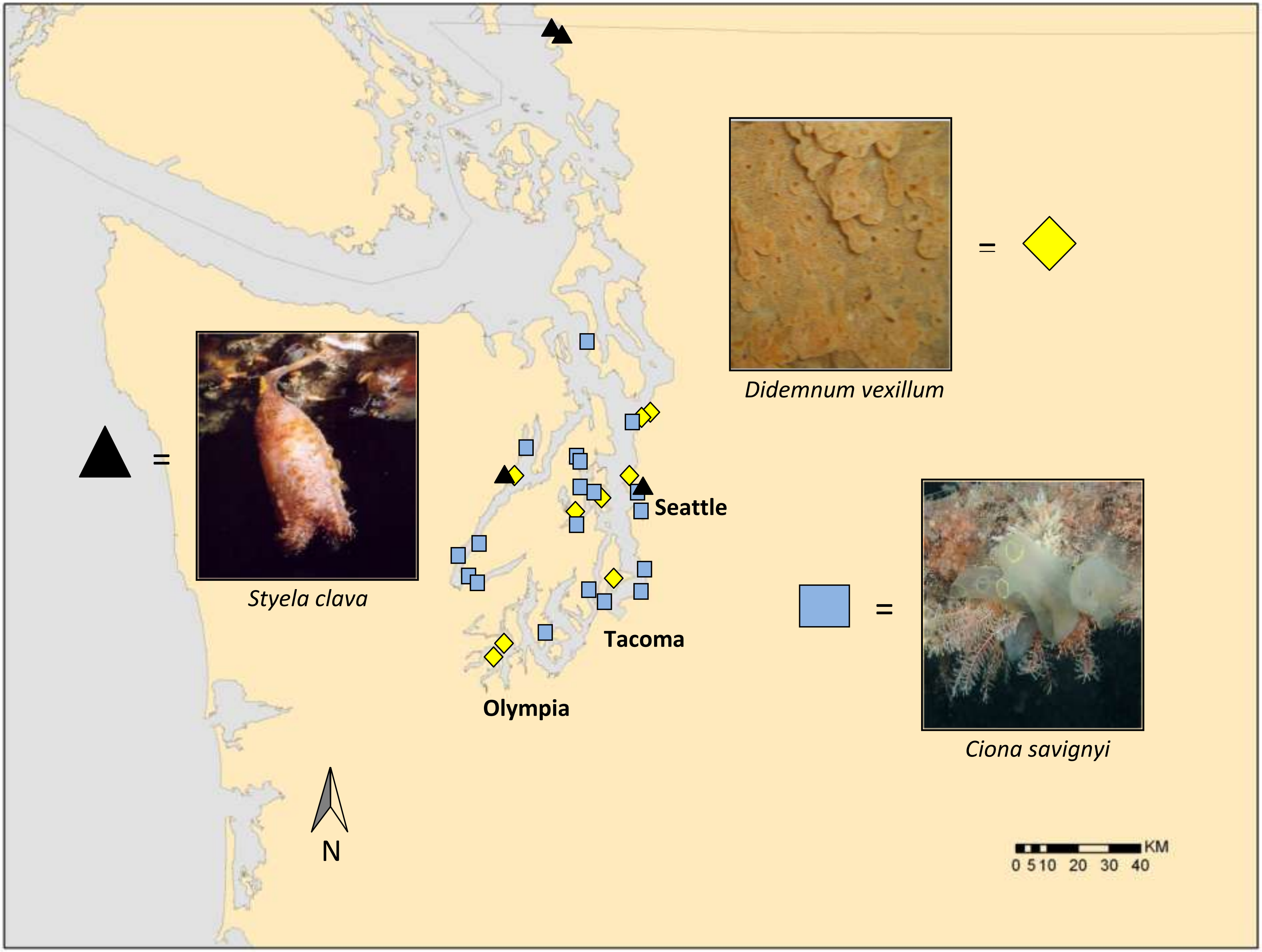

Didemnum vexillum
=
=
Seattle
Styela clava
=
Tacoma
Olympia
Ciona savignyi
N
KM
0 5 10 20 30 40

Plate 2

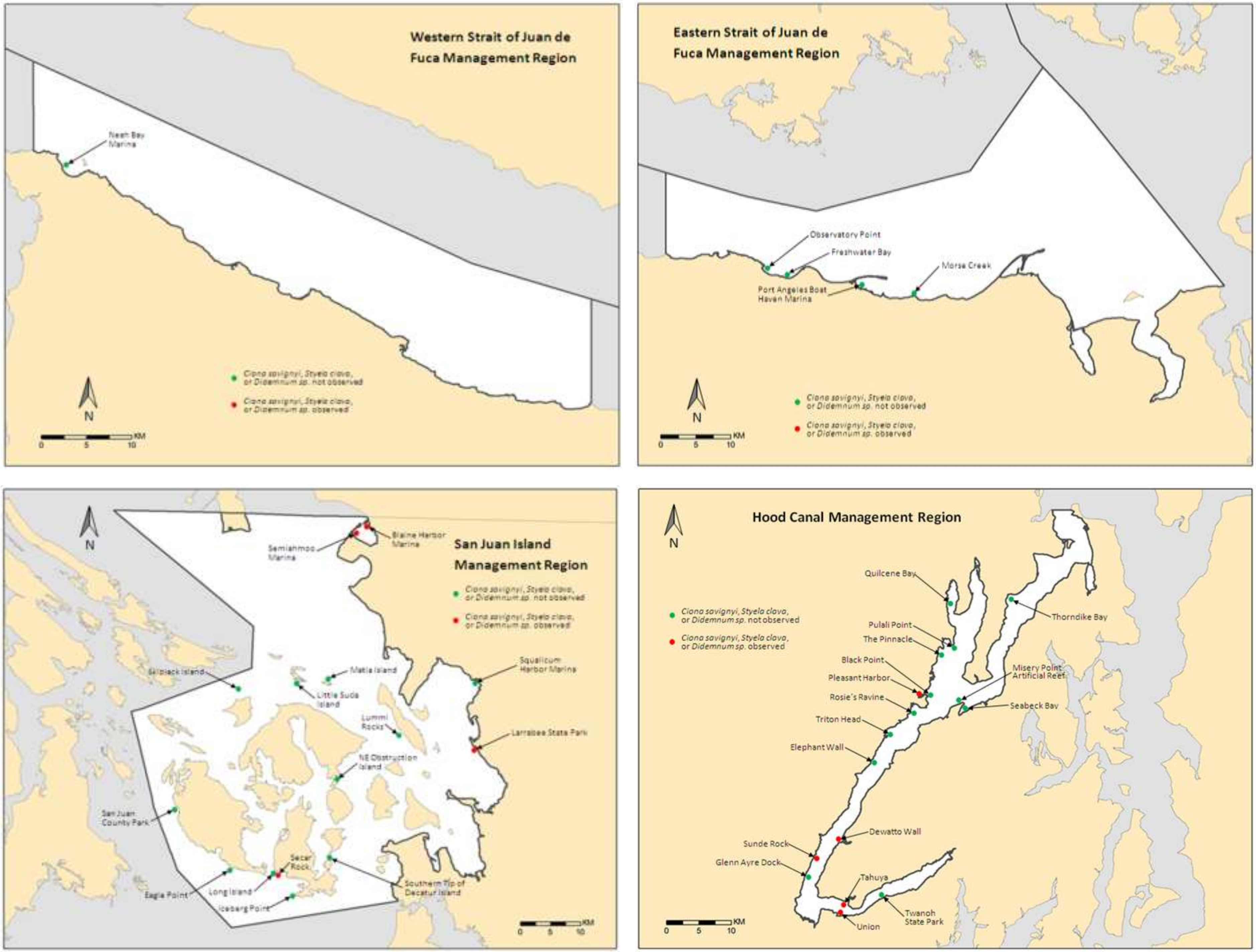
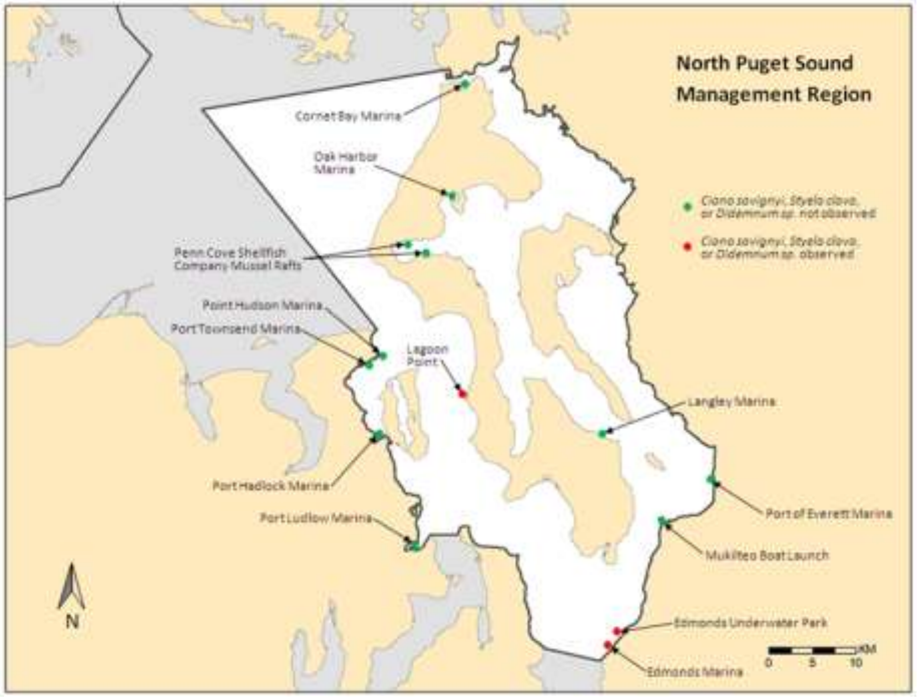
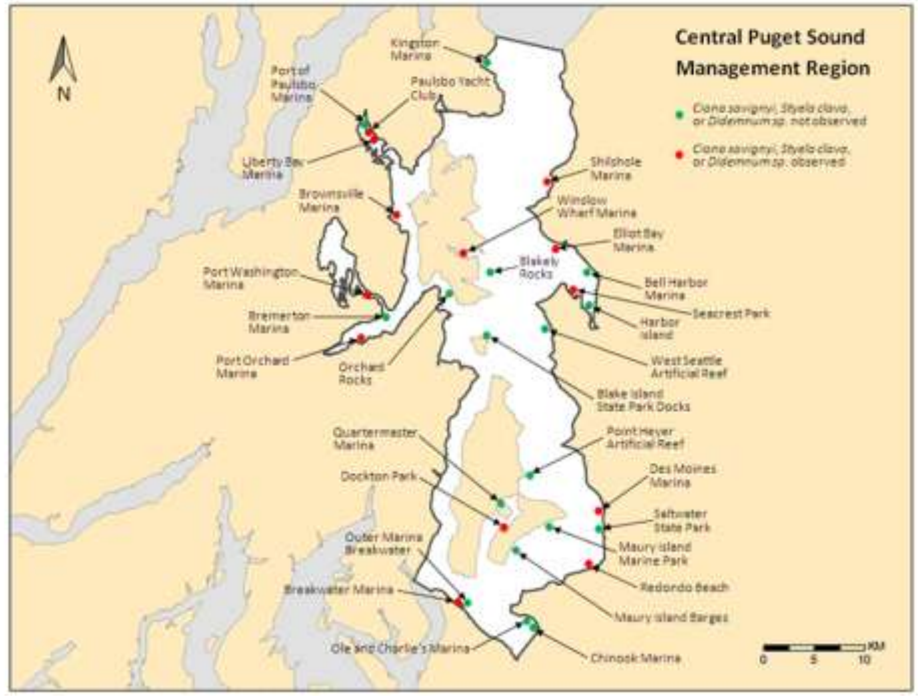

Western Strait of Juan de Fuca Management Region
Neah Bay Marina
Ciona savignyi, Styela clava, or Didemnum sp. not observed
Ciona savignyi, Styela clava, or Didemnum sp. observed
N
0 5 10 KM
Eastern Strait of Juan de Fuca Management Region
Observatory Point
Freshwater Bay
Port Angeles Boat Haven Marina
Morse Creek
Ciona savignyi, Styela clava, or Didemnum sp. not observed
Ciona savignyi, Styela clava, or Didemnum sp. observed
N
0 5 10 KM
San Juan Island Management Region
Ciona savignyi, Styela clava, or Didemnum sp. not observed
Ciona savignyi, Styela clava, or Didemnum sp. observed
N
Semiahmoo Marina
Blaine Harbor Marina
Matia Island
Little Sucia Island
Squalicum Harbor Marina
Lummi Rocks
Larrabee State Park
NE Obstruction Island
San Juan County Park
Secar Rock
Eagle Point
Long Island
Iceberg Point
Southern Tip of Decatur Island
0 5 10 KM
Hood Canal Management Region
N
Ciona savignyi, Styela clava, or Didemnum sp. not observed
Ciona savignyi, Styela clava, or Didemnum sp. observed
Quilcene Bay
Thorndike Bay
Pulali Point
The Pinnacle
Black Point
Misery Point Artificial Reef
Pleasant Harbor
Rosie's Ravine
Seabeck Bay
Triton Head
Elephant Wall
Dewatto Wall
Sunde Rock
Glenn Ayre Dock
Tahuya
Twanoh State Park
Union
0 5 10 KM

Plate 3


North Puget Sound
Management Region
Ciona savignyi, Styela clava, or Didemnum sp. not observed
Ciona savignyi, Styela clava, or Didemnum sp. observed
Cornet Bay Marina
Oak Harbor Marina
Penn Cove Shellfish Company Mussel Rafts
Point Hudson Marina
Port Townsend Marina
Lagoon Point
Langley Marina
Port Hadlock Marina
Port Ludlow Marina
Port of Everett Marina
Mukilteo Boat Launch
Edmonds Underwater Park
Edmonds Marina
N
0 5 10 KM


Central Puget Sound
Management Region
Ciona savignyi, Styela clava, or Didemnum sp. not observed
Ciona savignyi, Styela clava, or Didemnum sp. observed
N
Kingston Marina
Port of Poulsbo Marina
Poulsbo Yacht Club
Liberty Bay Marina
Brownsville Marina
Shilshole Marina
Winslow Wharf Marina
Elliot Bay Marina
Blakely Rocks
Port Washington Marina
Bell Harbor Marina
Seacrest Park
Harbor Island
Bremerton Marina
Port Orchard Marina
Orchard Rocks
West Seattle Artificial Reef
Blake Island State Park Docks
Point Heyer Artificial Reef
Quartermaster Marina
Des Moines Marina
Dockton Park
Saltwater State Park
Outer Marina Breakwater
Maury Island Marine Park
Redondo Beach
Breakwater Marina
Maury Island Barges
Ole and Charlie's Marina
Chinook Marina
0 5 10 KM

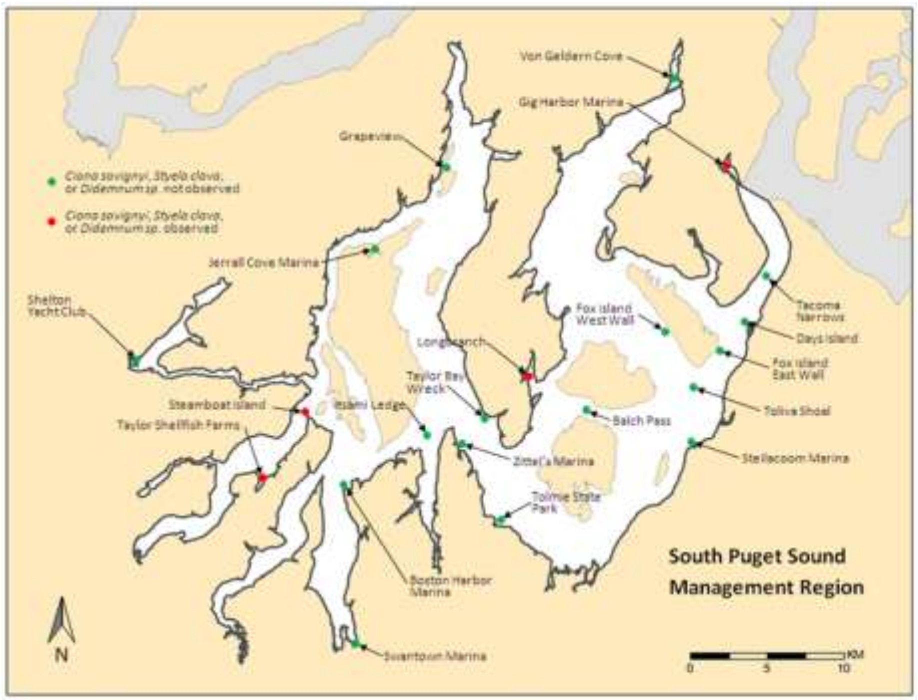

Von Geldern Cove
Gig Harbor Marina
Grapeview
Ciona savignyi, Styela clava, or Didemnum sp. not observed
Ciona savignyi, Styela clava, or Didemnum sp. observed
Jerrall Cove Marina
Shelton Yacht Club
Tacoma Narrows
Fox Island West Wall
Days Island
Longbranch
Fox Island East Wall
Taylor Bay Wreck
Steamboat Island
Itsami Ledge
Toliva Shoal
Taylor Shellfish Farms
Balch Pass
Zittel's Marina
Steilacoom Marina
Tolmie State Park
South Puget Sound
Management Region
Boston Harbor Marina
N
Swantown Marina
0 5 10 KM

Plate 4

***Didemnum vexillum* at Dockton Park**

Dockton Park is part of the King County Parks system and is located on the west side of Maury Island in the hydrographically defined Main Basin of Puget Sound and lies within the Central Puget Sound Management Region (see Plates 1 & 4). The docks are constructed of concrete pontoons; cover a surface area of approximately 934m^2; and are secured in place by 52 wood pilings. Depths under the docks range from approximately 3m - 6m (mean lower low water) and the seafloor beneath the docks is composed of mud and silt. An elevated pier connects the docks to the shore and a small concrete surfaced swim dock made buoyant by plastic pontoons is attached to the seaward end of the pier (Figure 1).

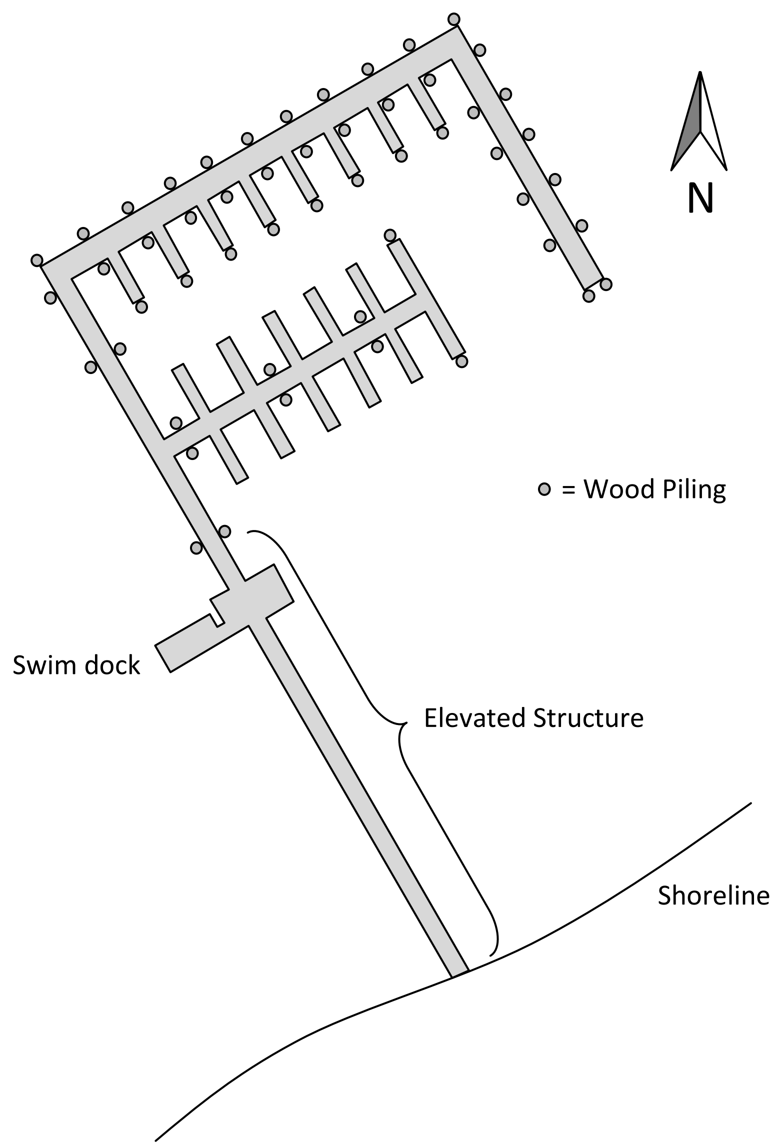


Figure 1. Schematic diagram of Dockton Park drawn to approximate scale.

In July 2007, WDFW received a citizen report that *D. vexillum* was growing on the undersides of the docks. In September 2007, biologists from the WDFW Marine Fish Program conducted a brief reconnaissance dive at the park and confirmed the infestation. In a follow-up dive survey by AISU biologists in November 2007, *D. vexillum* was found to be abundant and appeared to be uniformly distributed throughout the dock facility, though it was not present on the swim dock. It occurred almost exclusively on the casings of the clump-forming sabellid feather duster worm *Eudistylia vancouveri* which comprised the majority of the dock fouling biomass. Smaller *D. vexillum* colonies were observed attached directly to the sides and undersides of the docks and to the surrounding pilings.

In order to evaluate the local geographic extent of the infestation, AISU biologists conducted dive surveys of several nearby structures including 12 of approximately 30 mooring buoys located adjacent to the park docks; a boat ramp dock that is part of the park and located approximately 200m to the SW of the docks; a small private dock complex locally known as Plancich Dock located less than a quarter km NW of the park; several derelict pilings along the southern shore of the Dockton bight; and a small privately owned marina (Quartermaster Marina) located approximately 3.5 watercourse km NW of Dockton at the head of Quartermaster Harbor (Figure 2). Permission to enter an additional small marina owned by the Quartermaster Yacht Club, also located at the head of Quartermaster Harbor, could not be secured and it was not surveyed. Some large *D. vexillum* colonies were found at the Plancich Dock; however, no *D. vexillum* was found on any of the other structures examined.

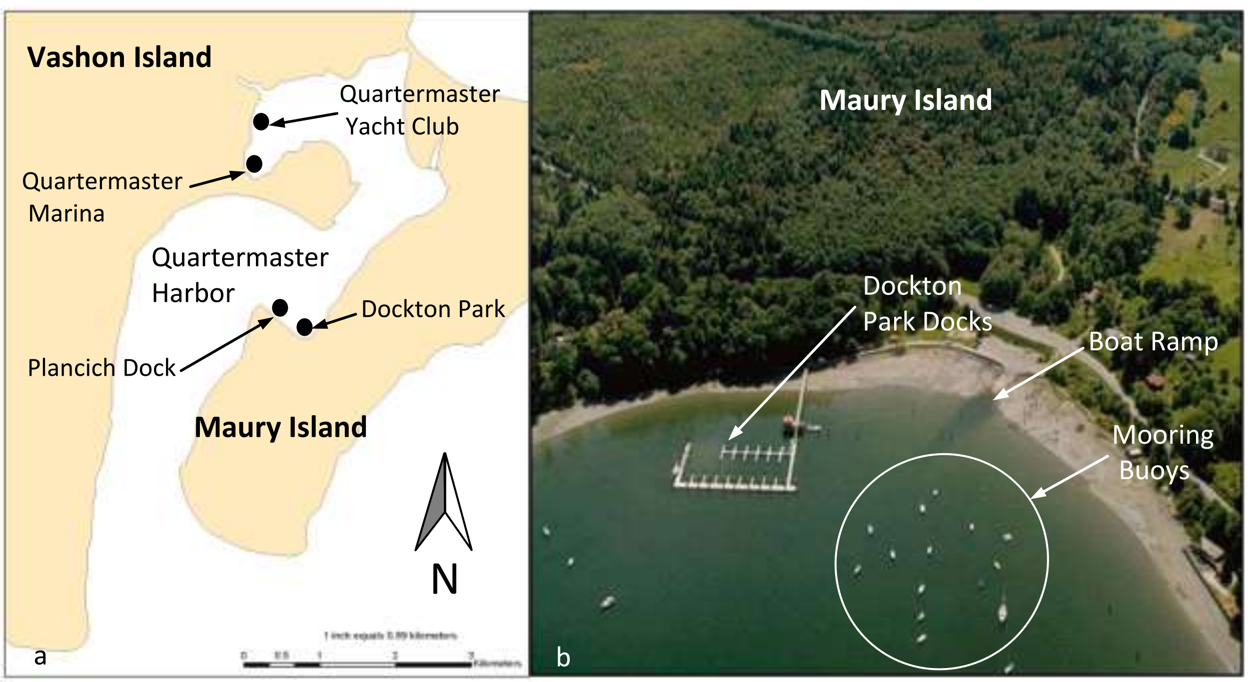


Figure 2. a) Location of Dockton Park and nearby surveyed facilities. b) Aerial view of Dockton Park.

Owing to the facilities small size, limited use during the winter, and relative insularity from other major marine structures, AISU biologists elected to use Dockton Park as an eradication methods evaluation and test site. Five treatment methods were chosen as follows:

1) Scrape and remove. All *D. vexillum* was scraped from the docks, placed in containers, and removed from the water for disposal at a land based refuge site;

2) Wrap with acetic acid infusion. Docks were wrapped in a polyethylene tarp, the ambient pH was lowered with a one-time infusion of 20% acetic acid, and the tarps were removed after 24 hours;

3) Wrap and leave. Docks were wrapped in a polyethylene tarp and left for a period of two weeks. Unexchanged, encapsulated water was rendered hypoxic as respiring dock fouling animals consumed the available oxygen;

4) Wrap with freshwater infusion. Docks were wrapped in a polyethylene tarp, the salinity was lowered with a one-time infusion of freshwater, and the tarps were removed after 24 hours; and

5) Tank sprayed acetic acid. Thirty percent acetic acid was sprayed directly onto the *D. vexillum* colonies, *in situ*, through a hose end sprayer attached to a pressurized reservoir.

In January, 2008, prior to the treatments, a presence/absence survey of the dock-fouling macrofauna taxa was conducted to test for community structure homogeneity within the facility and to ensure that any post-treatment effects on community structure could be reasonably attributed to the treatment and not to preexisting differences. The facility was mapped and overlaid with a $10m^2$ section grid. Thirty five of the sections contained dock structure and ten of the sections were randomly selected for macrofauna survey. Three randomly placed non-overlapping $1m^2$ quadrats were surveyed within each of the ten sections (Figure 3). Using hand-held lights, divers carefully examined all of the surface area within each quadrat and compiled a list of macrofauna species to the lowest practical taxonomic level. Highly mobile animals such as fish and shrimp were not included in the survey.

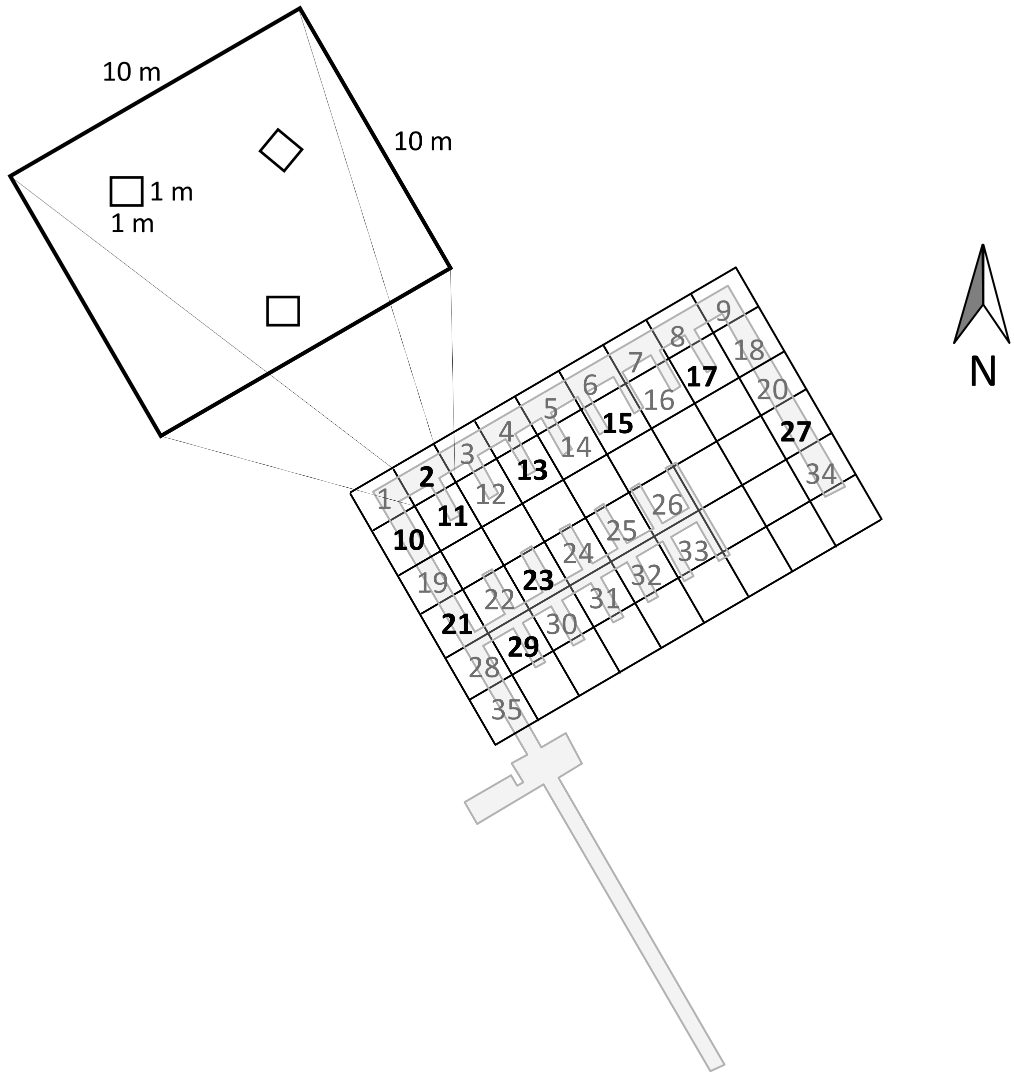


Figure 3. Sample section grid for Dockton Park showing the location of ten randomly selected sections (bold) that were surveyed for macrofauna including *D. vexillum* with 1m^2 quadrats.

Due to the growth habit and life history characteristics of *D. vexillum*, typical quantitative assessment methods based on metrics such as weight, area covered, counts, etc. proved problematic. These methods are particularly difficult to employ at Dockton Park, where the majority of *D. vexillum* colonies were spread on and around irregularly formed and variously sized clumps of tube worms. In an attempt to characterize the presence and distribution of *D. vexillum* at Dockton Park, we developed a rank order scale of abundance based on visually estimated percent coverage of the tube worm casings encountered within a quadrat as follows: 0 = no *D. vexillum* was observed; 1 = 25% coverage; 2 = 75% coverage; and 3 = 100% coverage (*D. vexillum* covered 100% of the worm casing surface area). Results of the pre-treatment macrofauna survey, including *D. vexillum* rank order scores, are summarized in Table 2. *Didemnum vexillum* was present in every quadrat surveyed and the mean rank order score expressed as percent coverage averaged over all 30 quadrats was 47%.

Table 2. Results of pre-treatment macrofauna presence/absence survey of 30 quadrats at Dockton Park.

Species	Section 2			Section 10			Section 11			Section 13			Section 15			Section 17			Section 21			Section 23			Section 27			Section 29		
	Quadrat 1	Quadrat 2	Quadrat 3	Quadrat 1	Quadrat 2	Quadrat 3	Quadrat 1	Quadrat 2	Quadrat 3	Quadrat 1	Quadrat 2	Quadrat 3	Quadrat 1	Quadrat 2	Quadrat 3	Quadrat 1	Quadrat 2	Quadrat 3	Quadrat 1	Quadrat 2	Quadrat 3	Quadrat 1	Quadrat 2	Quadrat 3	Quadrat 1	Quadrat 2	Quadrat 3	Quadrat 1	Quadrat 2	Quadrat 3
Didemnum Sp. (Colonial Tunicate)	2	2	2	1	1	1	2	2	1	2	1	1	2	2	1	1	1	2	1	1	1	2	1	1	1	1	2	1	2	1
Eudistylia vancouveri (Northern Feather Duster Worm)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Metridium senile (Sea Anemone)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hydroid (*Obelia Sp. ?*)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Balanus crenatus (Barnacle)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mytilus Sp. (Mussel)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓			✓	✓
Mopalia hindsii (Chiton)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pisaster ochraceus (Ochre Sea Star)	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓				✓		✓		✓
Hermissenda crassicornis (Opalescent Nudibranch)				✓															✓						✓		✓	✓		✓
Diaulula sandiegensis (Ringed Nudibranch)						✓																								
Aeolidia papillosa (Shaggy Mouse Nudibranch)						✓																								
Eupentacta quinquesemita (White Sea Cucumber)	✓		✓	✓		✓	✓	✓		✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓		✓	✓	✓		✓		✓
Cucumaria miniata (Orange Sea Cucumber)			✓	✓			✓	✓				✓			✓	✓	✓					✓		✓	✓	✓	✓	✓		
Cucumaria piperata (Speckled Sea Cucumber)			✓	✓		✓										✓	✓					✓				✓		✓		
Parastichopus californicus (California Sea Cucumber)				✓																										
Pododesmus cepio (Rock Oyster)	✓				✓		✓	✓	✓	✓	✓	✓		✓	✓		✓	✓	✓			✓		✓					✓	
Pugettia producta (Northern Kelp Crab)		✓								✓								✓			✓				✓		✓	✓		
Strongylocentrotus droebachiensis (Green Sea Urchin)			✓	✓			✓				✓		✓						✓				✓							

We converted *D. vexillum* rank order scores to presence/absence, combined the data from all three quadrats within each section, and used the Sørensen index of similarity to compare community structure among sections: $100 \cdot 2a/2a + b + c$ where a is the number of taxa present in both samples, b is the number of taxa present in sample 1 but absent from sample 2, and c is the number of taxa absent in sample 1 but present in sample 2. Index values ranged from 78 to 100 (mean = 88) indicating a high level of community structure similarity among sections and no apparent trend in spatial distribution across the facility (Table 3).

Table 3. Matrix of Sørensen's community similarity indices based on pre-treatment presence/absence surveys of macrofauna among ten randomly selected sections at Dockton Park.

Section	2	10	11	13	15	17	21	23	27
10	82.76								
11	91.67	81.48							
13	96.00	78.57	95.65						
15	91.67	81.48	100.00	95.65					
17	96.00	78.57	86.96	91.67	86.96				
21	88.00	78.57	86.96	91.67	86.96	83.33			
23	96.00	85.71	95.65	91.67	95.65	91.67	83.33		
27	88.00	78.57	78.26	83.33	78.26	91.67	83.33	83.33	
29	92.31	82.76	83.33	88.00	83.33	96.00	88.00	88.00	96.00

Upon completion of the pre-treatment survey, the finger docks were numbered and each of the five treatments was randomly assigned to four docks as follows: Docks 5, 9, 17, and 18 were treated with scrape and remove; docks 3, 7, 16, and 20 were treated using wrap with acetic acid infusion; docks 1, 2, 11, and 12 were treated using wrap and leave; docks 4, 6, 14, and 15 were treated using wrap with freshwater infusion; and docks 8, 10, 13, and 19 were treated using tank sprayed acetic acid.

Post-treatment and control surveys were conducted 15 days following the conclusion of each treatment. Two 1m^2 quadrats were randomly positioned on either lengthwise half of the dock and surveyed as described above for the pre-treatment macrofauna community structure survey. Additionally, a qualitative post-treatment assessment of each dock was conducted by paired divers swimming the length of the dock and noting any *D. vexillum* seen. The post-treatment quadrat survey results are summarized in Table 4. For each treated finger dock, a control quadrat was surveyed on the adjacent, non-treated main arterial dock near the juncture where the two docks meet (Figure 4). Control quadrats were used to ensure that the un-treated community composition and *D. vexillum* density did not substantially change either spatially or temporally over the course of the treatments. The mean Sørensen similarity value averaged over all 20 control quadrats was high (86.50), as was the mean value of the control quadrats compared to the pre-treatment values presented in Table 3 (85.26). Thus, the un-treated community composition and *D. vexillum* density remained reasonably homogenous, and the overall community structure did not appreciably change during the treatment period.

To minimize the likelihood of dispersion through fragmentation and gamete release that may occur when colonies are disrupted, the treatments were conducted in January and February, when conditions are less amenable to spawning and astogenetic growth. Additionally, microscopic examination of samples from several colonies taken at the time of the treatments failed to find any indication that spawning was imminent or occurring.

Table 4. Results of post-treatment macrofauna presence/absence survey at Dockton Park including control quadrats.

Scrape and Remove

Species	Dock #5 Quadrat 1	Dock #5 Quadrat 2	Dock #5 Control	Dock #8 Quadrat 1	Dock #8 Quadrat 2	Dock #8 Control	Dock #17 Quadrat 1	Dock #17 Quadrat 2	Dock #17 Control	Dock #18 Quadrat 1	Dock #18 Quadrat 2	Dock #18 Control
Didemnum Sp. (Colonial Tunicate)	0	0	2	0	0	2	0	0	1	0	0	2
Eudistylia vancouveri (Northern Feather Duster Worm)			✓			✓			✓			✓
Metridium senile (Sea Anemone)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hydroid (Obelia Sp. ?)			✓	✓		✓			✓	✓	✓	✓
Balanus crenatus (Barnacle)		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓
Mytilus Sp. (Mussel)			✓	✓	✓	✓	✓			✓		✓
Mopalia hindsii (Chiton)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pisaster ochraceus (Ochre Sea Star)		✓			✓	✓	✓		✓	✓		✓
Hermissenda crassicornis (Opalescent Nudibranch)						✓				✓		✓
Diaulula sandiegensis (Ringed Nudibranch)												
Aeolidia papillosa (Shaggy Mouse Nudibranch)												
Eupentacta quinquesemita (White Sea Cucumber)			✓					✓	✓			✓
Cucumaria miniata (Orange Sea Cucumber)												
Cucumaria piperata (Speckled Sea Cucumber)			✓					✓	✓		✓	✓
Parastichopus californicus (California Sea Cucumber)												
Pododesmus cepio (Rock Oyster)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Pugettia producta (Northern Kelp Crab)												
Strongylocentrotus droebachiensis (Green Sea Urchin)												

Wrap with Acetic Acid Infusion

Species	Dock #3 Quadrat 1	Dock #3 Quadrat 2	Dock #3 Control	Dock #7 Quadrat 1	Dock #7 Quadrat 2	Dock #7 Control	Dock #16 Quadrat 1	Dock #16 Quadrat 2	Dock #16 Control	Dock #20 Quadrat 1	Dock #20 Quadrat 2	Dock #20 Control
Didemnum Sp. (Colonial Tunicate)	0	0	1	0	0	1	0	0	2	0	0	2
Eudistylia vancouveri (Northern Feather Duster Worm)			✓			✓			✓			✓
Metridium senile (Sea Anemone)	✓		✓			✓			✓			✓
Hydroid (Obelia Sp. ?)			✓			✓			✓			✓
Balanus crenatus (Barnacle)			✓			✓			✓			✓
Mytilus Sp. (Mussel)			✓			✓			✓			✓
Mopalia hindsii (Chiton)						✓			✓			✓
Pisaster ochraceus (Ochre Sea Star)			✓						✓			✓
Hermissenda crassicornis (Opalescent Nudibranch)									✓			
Diaulula sandiegensis (Ringed Nudibranch)												
Aeolidia papillosa (Shaggy Mouse Nudibranch)												
Eupentacta quinquesemita (White Sea Cucumber)			✓						✓			✓
Cucumaria miniata (Orange Sea Cucumber)									✓			
Cucumaria piperata (Speckled Sea Cucumber)												
Parastichopus californicus (California Sea Cucumber)												
Pododesmus cepio (Rock Oyster)							✓					✓
Pugettia producta (Northern Kelp Crab)												
Strongylocentrotus droebachiensis (Green Sea Urchin)												

Wrap and Leave

Species	Dock #1 Quadrat 1	Dock #1 Quadrat 2	Dock #1 Control	Dock #2 Quadrat 1	Dock #2 Quadrat 2	Dock #2 Control	Dock #11 Quadrat 1	Dock #11 Quadrat 2	Dock #11 Control	Dock #12 Quadrat 1	Dock #12 Quadrat 2	Dock #12 Control
Didemnum Sp. (Colonial Tunicate)	0	0	2	0	0	2	0	0	2	0	0	2
Eudistylia vancouveri (Northern Feather Duster Worm)			✓			✓			✓			✓
Metridium senile (Sea Anemone)	✓	✓	✓	✓		✓			✓	✓	✓	✓
Hydroid (Obelia Sp. ?)			✓			✓			✓			✓
Balanus crenatus (Barnacle)			✓			✓			✓			✓
Mytilus Sp. (Mussel)		✓				✓			✓	✓	✓	✓
Mopalia hindsii (Chiton)		✓	✓			✓			✓			✓
Pisaster ochraceus (Ochre Sea Star)			✓			✓						✓
Hermissenda crassicornis (Opalescent Nudibranch)									✓			✓
Diaulula sandiegensis (Ringed Nudibranch)												
Aeolidia papillosa (Shaggy Mouse Nudibranch)			✓									
Eupentacta quinquesemita (White Sea Cucumber)			✓			✓						✓
Cucumaria miniata (Orange Sea Cucumber)												
Cucumaria piperata (Speckled Sea Cucumber)			✓									
Parastichopus californicus (California Sea Cucumber)												
Pododesmus cepio (Rock Oyster)			✓			✓			✓			
Pugettia producta (Northern Kelp Crab)												
Strongylocentrotus droebachiensis (Green Sea Urchin)												

Wrap with Freshwater Infusion

Species	Dock #4 Quadrat 1	Dock #4 Quadrat 2	Dock #4 Control	Dock #6 Quadrat 1	Dock #6 Quadrat 2	Dock #6 Control	Dock #14 Quadrat 1	Dock #14 Quadrat 2	Dock #14 Control	Dock #15 Quadrat 1	Dock #15 Quadrat 2	Dock #15 Control
Didemnum Sp. (Colonial Tunicate)	1	1	2	1	1	1	1	1	1	1	1	1
Eudistylia vancouveri (Northern Feather Duster Worm)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Metridium senile (Sea Anemone)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hydroid (Obelia Sp. ?)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Balanus crenatus (Barnacle)	✓	✓			✓	✓	✓	✓			✓	✓
Mytilus Sp. (Mussel)	✓		✓		✓		✓	✓	✓	✓	✓	✓
Mopalia hindsii (Chiton)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pisaster ochraceus (Ochre Sea Star)	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	
Hermissenda crassicornis (Opalescent Nudibranch)												
Diaulula sandiegensis (Ringed Nudibranch)												✓
Aeolidia papillosa (Shaggy Mouse Nudibranch)												✓
Eupentacta quinquesemita (White Sea Cucumber)	✓	✓	✓	✓	✓	✓			✓	✓	✓	
Cucumaria miniata (Orange Sea Cucumber)				✓							✓	
Cucumaria piperata (Speckled Sea Cucumber)				✓			✓			✓	✓	
Parastichopus californicus (California Sea Cucumber)												
Pododesmus cepio (Rock Oyster)					✓	✓	✓	✓			✓	
Pugettia producta (Northern Kelp Crab)				✓				✓				
Strongylocentrotus droebachiensis (Green Sea Urchin)						✓						

Tank Sprayed Acetic Acid

Species	Dock #8 Quadrat 1	Dock #8 Quadrat 2	Dock #8 Control	Dock #10 Quadrat 1	Dock #10 Quadrat 2	Dock #10 Control	Dock #13 Quadrat 1	Dock #13 Quadrat 2	Dock #13 Control	Dock #19 Quadrat 1	Dock #19 Quadrat 2	Dock #19 Control
Didemnum Sp. (Colonial Tunicate)	1	1	2	1	1	1	1	1	2	1	1	2
Eudistylia vancouveri (Northern Feather Duster Worm)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Metridium senile (Sea Anemone)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hydroid (Obelia Sp. ?)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Balanus crenatus (Barnacle)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mytilus Sp. (Mussel)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mopalia hindsii (Chiton)	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Pisaster ochraceus (Ochre Sea Star)	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓
Hermissenda crassicornis (Opalescent Nudibranch)												
Diaulula sandiegensis (Ringed Nudibranch)												
Aeolidia papillosa (Shaggy Mouse Nudibranch)												✓
Eupentacta quinquesemita (White Sea Cucumber)	✓	✓	✓	✓	✓		✓	✓		✓	✓	
Cucumaria miniata (Orange Sea Cucumber)		✓		✓						✓	✓	
Cucumaria piperata (Speckled Sea Cucumber)	✓		✓					✓				✓
Parastichopus californicus (California Sea Cucumber)												
Pododesmus cepio (Rock Oyster)	✓	✓	✓	✓	✓			✓		✓	✓	✓
Pugettia producta (Northern Kelp Crab)		✓									✓	
Strongylocentrotus droebachiensis (Green Sea Urchin)	✓			✓	✓	✓						

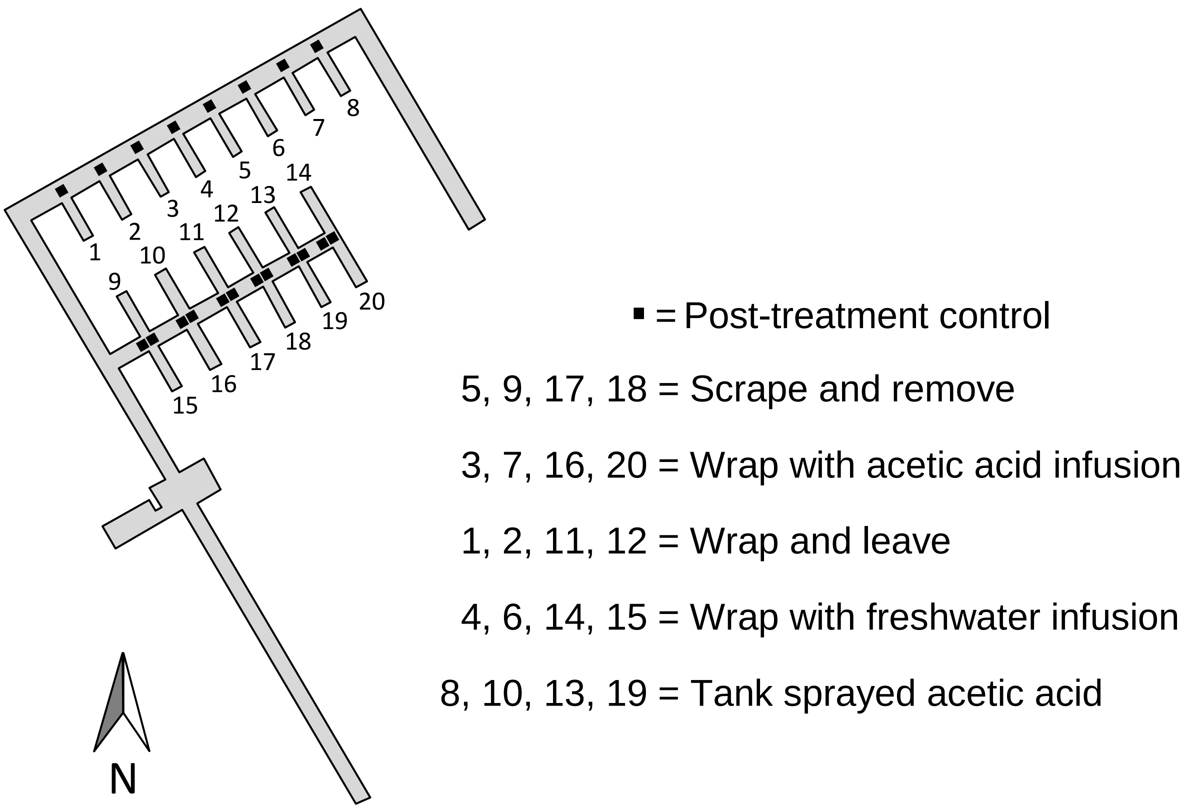


Figure 4. Numbered finger docks at Dockton Park and their randomly assigned treatments, and locations of post-treatment control quadrats.

Scrape and Remove

On January 30, AISU biologists removed all of the visible *D. vexillum* colonies from docks 5, 9, 17, and 18. Initial efforts to remove the colonies from individual worm casings proved to be prohibitively time consuming and resulted in incomplete removal of the colonies. In order to ensure complete removal of all *D. vexillum*, infested clumps of feather duster worms were contained in their entirety in plastic bags and scraped from the dock. The bags were passed to the surface, and subsequently disposed of in a landfill. *Didemnum vexillum* colonies that were attached directly to the dock or to other hard surfaces were scraped off and similarly disposed of. A total of 208Kg of biotic material including *D. vexillum* was removed.

No *D. vexillum* colonies were found during the post-treatment survey. An examination of the post-treatment community structure data indicates that those animals that were found to associate most closely with the feather duster worm clumps (e.g. sea cucumbers, nudibranchs, kelp crabs) during the pre-treatment survey were not present post-treatment and were likely removed along with the worm colonies. The presence of animals that tended not to use the worm clumps directly as refuge, substrate, or food appeared to be unaffected (e.g. chitons, rock oysters, barnacles) (Table 4).

Wrap with Acetic Acid Infusion

On February 11, docks 3, 7, 16, and 20 were wrapped and treated with 20% acetic acid. Divers and surface support personnel wrapped a single pre-cut opaque polyethylene tarp around each of the four docks. The tarps were cinched and secured with rope and waterproof PVC tape to ensure a tight fit and to reduce the volume of encapsulated water, and to minimize the exchange of encapsulated water with the ambient seawater. Acetic acid was hand pumped through two hoses simultaneously from a single container and introduced in equal volumes through four slits (two on each side of the dock) cut above the water line. The slits were sealed at the conclusion of the treatment (Figure 5).

Figure 5. a) SCUBA divers and surface personnel encapsulating a dock with polyethylene plastic. b) Pumping 20% acetic acid into the encapsulation. c) Completed wrap with acetic acid infusion.

The volume of encapsulated water was estimated to be 3400L per dock. Each dock was infused with 170L of 20 % acetic acid to achieve a final estimated concentration of 5% (50L acetic acid/1000L of seawater). A one L sample of water was drawn through each of the four slits 10 minutes, 30 minutes, and 24 hours after the infusion was completed. The wrap was removed after 24 hours. The water samples from each timed interval were combined, measured for pH, and compared to an ambient non-treated 4L sample drawn from outside the enclosure (pH 6.78). The pH of the treated water remained highly acidic throughout the 24 hour treatment period (Table 5). The post-treatment survey revealed that nearly all of the dock fouling biomass, including *D. vexillum*, had been exterminated (Table 4).

Table 5. Timed interval post-treatment pH.

Time	**10min**	**30min**	**24hrs**
Dock #	**pH**		
3	3.71	3.72	4.79
7	4.14	4.06	4.92
16	4.07	3.9	6.08
20	4.46	4.38	6.08

Wrap and Leave

On February 12, docks 1, 2, 11, and 12 were wrapped with polyethylene as described above for the wrap with acetic acid infusion treatment. The docks were left wrapped and undisturbed for two weeks. Immediately upon removal of the tarp, divers conducted a qualitative survey of the bottoms of the docks and the seafloor beneath. None of the dock fouling biota appeared to have survived. The feather duster worms had either partially or completely dislodged from their casings while the casings remained intact and firmly attached to the bottom of the dock. *Didemnum vexillum* appeared to be present; however, tactile examination of the tunic material revealed that it was very loosely attached and it quickly disintegrated when handled, indicating that the zooids had not survived the treatment and that the tunic was decomposing (Figure 6a). Nearly all of the remaining biota was detached from the dock and had fallen to the seafloor (Figure 6b). An offensive odor generated from accumulated ammonia and sulphide was released upon removal of the tarp, indicating an advanced stage of hypoxia within the encapsulation; however, the biota did not appear to be decayed, which suggests that the water had only recently become lethally hypoxic, or that the severe hypoxia had delayed the onset of decomposition.

Figure 6. a) Lethally suffocated colonies of *Didemnum vexillum* and feather duster worms (*Eudistylia vancouveri*) from a wrap and leave treated dock at Dockton Park. b) Seafloor beneath a wrap and leave treated dock at Dockton Park showing lethally suffocated biota that detached from the dock undersurface.

The 15 days post-treatment survey revealed that nearly all of the fouling biomass had been exterminated and mortality among *D. vexillum* colonies appeared to be 100% (Table 3). Though a small number of mussels (*Mytilus sp.*) and sea anemones (*Metridium senile*) were noted during the post-treatment survey, it was not determined whether these animals were living or had become deceased and failed to detach.

Wrap with Freshwater Infusion

On February 14, docks 4, 6, 14, and 15 were wrapped as previously described and infused with freshwater. Freshwater was pumped through two hoses simultaneously from a single spigot and introduced in equal volumes through four slits as described for the wrap with acetic acid infusion treatment. Freshwater was introduced for 1.5 hours at a rate of approximately 10.5L per minute for a total infusion of 945L.

The post-treatment survey results indicate that the treatment had little or no effect on the macrofauna assemblage, including *D. vexillum* (Table 3). Ambient and post-treatment salinity measurements were not made; however, the pH measurements made during the wrap with acetic acid infusion treatment suggest that exchange of the encapsulated water with outside seawater over a 24 hour period would have been minimal. An introduction of approximately one third by volume freshwater should have significantly lowered the salinity within the encapsulation. Though it does appear that *D. vexillum* is resilient to at least short term exposure to lower than ambient salinities at this location, a critical salinity threshold that was not achieved during this treatment may have produced more favorable results. Many of the other dock-fouling organisms at this site (barnacles, kelp crabs, chitons, rock oysters, etc.) are known to have high tolerances to low salinities, thus lack of mortality among these animals offers little insight into how much qualitative change occurred to the encapsulated water at the prescribed infusion rate and treatment duration.

Tank Sprayed Acetic Acid

On February 15, docks 8, 10, 13, and 19 were treated with tank sprayed acetic acid. A 12 cubic foot SCUBA cylinder with regulator was used to pressurize a conventional 12L garden-style hose-end sprayer filled with 30% acetic acid (Figure 7). The nozzle was adjusted for a medium spray pattern and directed *in situ* onto the surface of the *D. vexillum* colonies from a distance of 10-20cm. The application rate was approximately 20L per dock. The acetic acid solution is presumed to be instantaneously diluted as it leaves the nozzle, therefore any exposure to lowered pH would be very short-lived.

Figure 7. Hose end sprayer pressurized with SCUBA cylinder used for in situ application of 30% acetic acid.

The post-treatment survey results indicate that the treatment may have been partially effective, as the rank order scores from the treated quadrats were generally lower than those recorded from the adjacent control quadrats, and some *D. vexillum* mortality was noted during the qualitative assessment; however, many living colonies of *D. vexillum* remained. None of the other dock fouling organisms appeared to have been affected (Table 4).

Cost Per Treatment

An estimate of the cost per treatment at Dockton Park along with an effectiveness score based on the apparent success or failure of each treatment are presented in Table 6. In order to provide an equitable assessment of the relative cost for each treatment, the total cost column reflects only those dollar amounts associated with the purchase of consumable materials and disposal fees. It does not include the cost of reusable items such as garden hoses, pumps, and containers, or ancillary costs associated with equipment staging and transportation. Since the wages for participating personnel on this project varied widely and do not necessarily reflect the labor costs that would be incurred during a routine application, total personnel hours rather than a dollar amount based on labor cost per unit time are reported (i.e. wages are not included in the total cost).

In terms of both personnel hours and material/disposal cost, scrape and remove proved to be the least expensive eradication option at this location. Acetic acid infusion and wrap and leave were highly effective, but far more costly and would likely pose considerable logistical challenges at high occupancy dock facilities such as large marinas. Economies of scale and mitigating factors such as ease of access, dock configurations, structural materials, etc. have not been evaluated and may substantially change the relative materials and labor costs. Thus, extrapolating these estimates to other facilities and structures may not be appropriate at this time.

Table 6. Relative cost, effort, and effectiveness of five *Didemnum vexillum* eradication treatments at Dockton Park. Effectiveness scores are as follows: 1 = apparent complete eradication; 2 = apparent partial eradication; 3 = no apparent effect.

Treatment Method	Personnel Hours	Area (m^2)	Material/ Disposal Cost	Effectiveness
Scrape and remove	8.8	44.6	$27.00	1
Wrap with acetic acid infusion	11.2	44.6	$706.00	1
Wrap and leave	9.2	44.6	$198.00	1
Wrap with freshwater infusion	21.2	44.6	$198.00	3
Tank sprayed acetic acid	5.2	44.6	$88.00	2

Survival of Detached *Didemnum vexillum* Colonies

During preliminary dives at Dockton, biologists noted that feather duster worm casing clumps that had become dislodged from the docks and settled on the seafloor were not infested with *D. vexillum*. The clumps likely became dislodged during storms or when the weight of the worm colony exceeded its capacity to remain attached, possibly compounded by the added weight of *D. vexillum*. In order to test the ability of *D. vexillum* to survive a benthic existence at this location, on November 14, 2007, five large, heavily infested (rank order score = 3) worm clumps were manually detached from the dock underside and allowed to fall to the bottom. Each clump was tethered to the seafloor with a 3m length of string fastened to a labeled (A-D) steel stake (Figure 8). Tethering the clumps to the seafloor enabled divers to easily relocate them in limited visibility while, at the same time, permitting the clumps to assume whatever physical relation to the seafloor that would otherwise have occurred as a result of storm surge, tidal current, etc..

No *D. vexillum* (rank order score = 0) was found on any of the clumps 22 and 43 days after detachment. *Didemnum vexillum* has not been known to inhabit soft-bottom habitats and results from this detachment experiment are consistent with observations of colonies that were dislodged from a barge in New Zealand, where colonies that settled on hard substrate tended to survive while those that settled on muddy or sandy bottoms eventually died. At Dockton Park, though the worm tube parchment remained largely intact, most of the worms were no longer present. Possible explanations for the lack of survival of *D. vexillum* colonies that have come into direct contact with the seafloor at Dockton Park include increased susceptibility to predation and/or inhibited feeding and circulation.

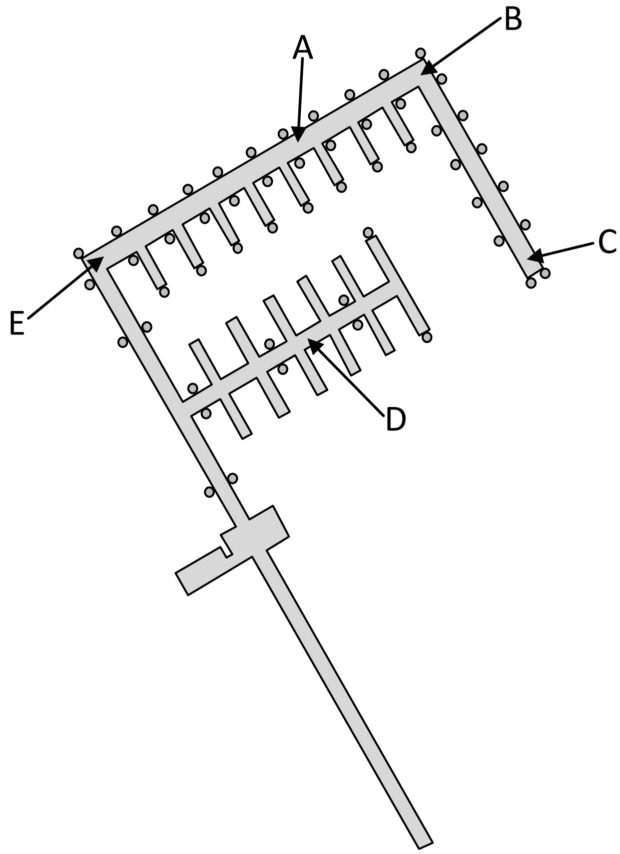


Figure 8. Location of five detached *Didemnum vexillum* infested feather duster worm clumps that were used to test the ability of *D. vexillum* to survive on the seafloor at Dockton Park.

Pilings Treated with Tank Sprayed Acetic Acid

The apparent partial success of tank sprayed acetic acid on the four treated docks and its ease of application prompted further investigation using *D. vexillum* colonies that inhabited the pilings at Dockton Park. All of the pilings were surveyed for *D. vexillum* presence/absence and 21 of the 52 pilings were found to have some colonization. When present, the colonies appeared to be restricted to that segment of the pilings that occurred below the low water mark and from 1-2m above the seafloor. Two colonized pilings were randomly selected for treatment. Visual estimates of colony sizes on four neighboring pilings were noted and served as references (Figure 9).

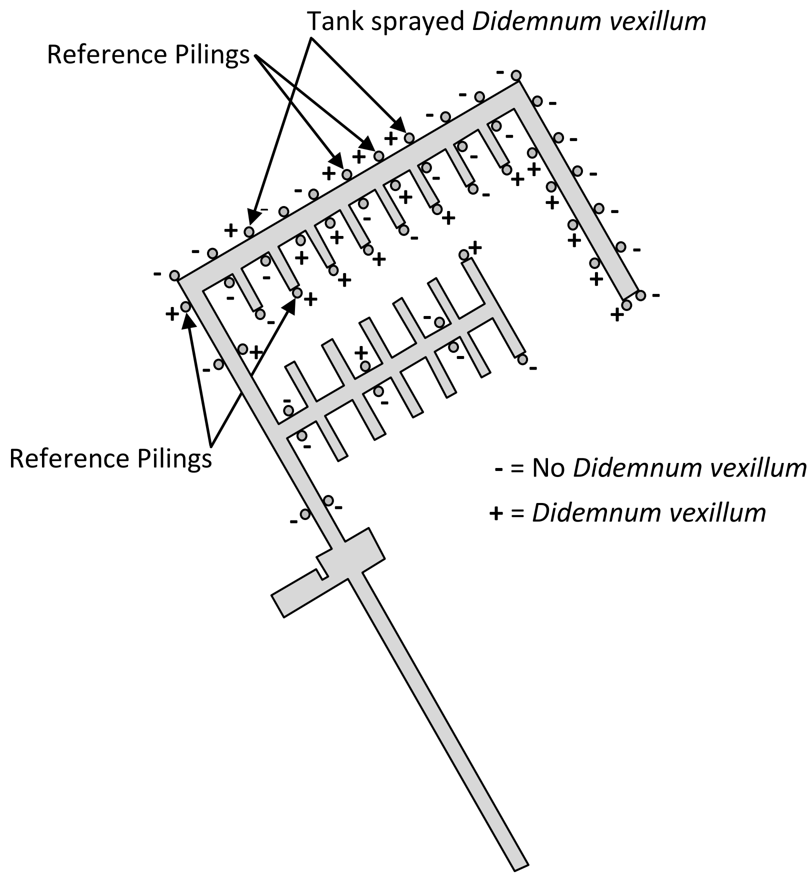


Figure 9. Results from the presence/absence survey for *Didemnum vexillum* on 52 wood pilings at Dockton Park, and the location of two tank sprayed acetic acid treatment pilings and four reference pilings.

Thirty percent acetic acid was applied to the colonies at a rate of approximately 1L/m^2 on May 28, 2008. After 20 days, the treated colonies were re-examined and the area covered was estimated to have been reduced by approximately 50% (Figure 10). No detectable reduction in colony size was noted on any of the reference pilings.

Figure 10. Colony of *Didemnum vexillum* treated with tank sprayed acetic acid. a) 30% acetic acid being applied to the colony through a hose end sprayer. b) Same colony immediate post treatment. c) Same colony twenty days post treatment. Circles denote photo landmarks.

Final *Didemnum vexillum* Removal

During May 19-21, 2008, after the conclusion of the eradication methods test, the AISU contracted with a local commercial dive company (Ballard Diving and Salvage, Inc.) to remove all of the *D. vexillum* from the docks at Dockton Park. Surface support and disposal arrangements were provided by AISU staff. The divers scraped all encountered colonies from the dock undersides and, when colonies inhabited feather duster worm clumps, the entire clump was removed from the dock. The material was placed in mesh bags underwater and passed to the surface where it was transferred to plastic bags and hauled upland to a dumpster. Nearly ten tons of biotic material was removed from the docks and subsequently deposited in a landfill (Figure 11). On May 28, AISU divers finished the removal by scraping all *D. vexillum* colonies from the pilings. On June 16, they returned to the park for a sweep of the facility and removed a few small colonies that had previously been overlooked.

Figure 11. *Didemnum vexillum* removal and disposal at Dockton Park.

On September 15, 2008, 91 days after the completion of the last removal sweep, AISU divers conducted a follow-up qualitative survey of the docks. They found that the undersides of those docks that had been treated with either wrap and leave or wrap with acetic acid infusion were nearly completely covered with mussels (*Mytilus sp.*) (Figure 12). Those docks that had been treated otherwise harbored a more diverse assemblage of species. Several small (< $10cm^2$) resurgent colonies of *D. vexillum* were found growing primarily on the backs of chitons in the form of short pendulous ropes or interspersed within the mussel beds as small mat forming colonies. On October 18, and November 18 and 24, AISU divers completed additional sweeps through the facility and, again, removed any colonies they encountered. On March 10, 2009, a brief examination of some of the docks and pilings revealed additional resurgent *D. vexillum* colonies. One such colony, depicted in Figure 13, exemplifies the rapid growth rates that *D. vexillum* can achieve. It is highly doubtful that a colony as large as the one shown was overlooked during previous removal sweeps. More likely, the colony was generated from an imperceptibly small "nucleus" of living tunicate that was not removed. If so, the colony grew from not visible to the size shown in about 3.5 to 5 months.

Figure 12. Underside of a wrap and leave treated dock (dock 1) at Dockton Park 91 days post-treatment showing near monotypic recolonization by the mussel *Mytilus sp.*.

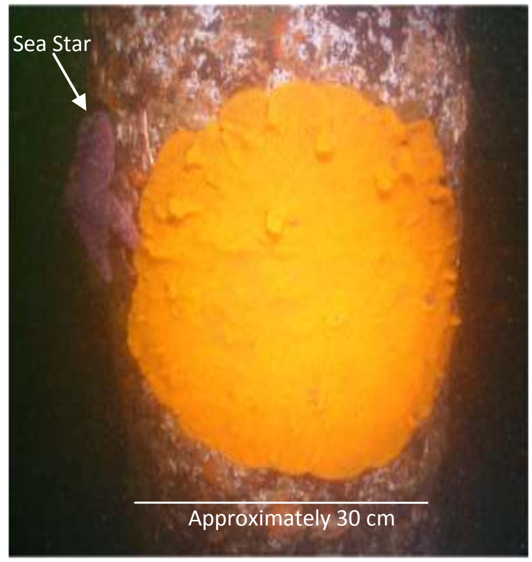


Figure 13. Resurgent colony of *Didemnum vexillum* on a piling at Dockton Park.

Plancich Dock

On April 20, 2009, the AISU contracted with Ballard Diving and Salvage, Inc. to remove all *D. vexillum* colonies from the privately owned dock complex located NW of Dockton Park (Figure 14). A staffed surface support vessel and disposal of the removed *D. vexillum* was provided by the AISU. As at Dockton Park, much of the *D. vexillum* occurred on clump-forming feather duster worms and all infested clumps were bagged and removed in their entirety. In addition, large rope-forming colonies were also attached to mooring lines and chains and some mat-forming colonies were attached directly to the underside of the docks. These were carefully scraped and removed for disposal. Approximately 181Kg of biotic material was removed from the complex and disposed of at a land based refuge site.

Figure 14. Aerial view of Plancich dock complex.

Pleasant Harbor

Pleasant Harbor is a small bay located just south of Quilcene Bay along the western shore of the hydrographically defined Hood Canal Basin. There are two privately managed marinas (Pleasant Harbor and Home Port) and a small state park dock located on the north shore of the bay. Nine small private residence docks are located within the bay – six along the south shore and three on the north shore. A recently installed WDFW public access boat ramp dock is located near the head of the bay (Figure 15). *Styela clava* was first reported in Pleasant Harbor in 2005 and to date, control efforts have been limited to annual hand removal of S. clava from all vessel hulls within the bay.

Figure 15. Aerial photograph of Pleasant Harbor.

The Pleasant Harbor Marina docks cover a surface area of approximately 6061 m^2 and are constructed primarily of concrete pontoons with some polystyrene pontoons and foam-filled tire floatation (Docks D, E, F, and I). There are approximately 17 covered slips. The docks are secured in place with both concrete and wood pilings (Figure 16). The marina and adjacent property was recently purchased by a Canadian based development company and a master-planned community is slated for ground breaking in 2009. The development project includes replacement and reorganization of the marina dock facilities and upland marina structures. In February and March, 2009, docks D, E, and F were replaced with plastic pontoon supported docks, and the adjacent wood pilings were replaced with steel pilings. The old docks and pilings were towed to a private beach near the mouth of the bay where they were removed from the water, dismantled, and hauled to an upland staging area to await disposal (Figure 17). Further dock replacements are likely to occur as the development project progresses.

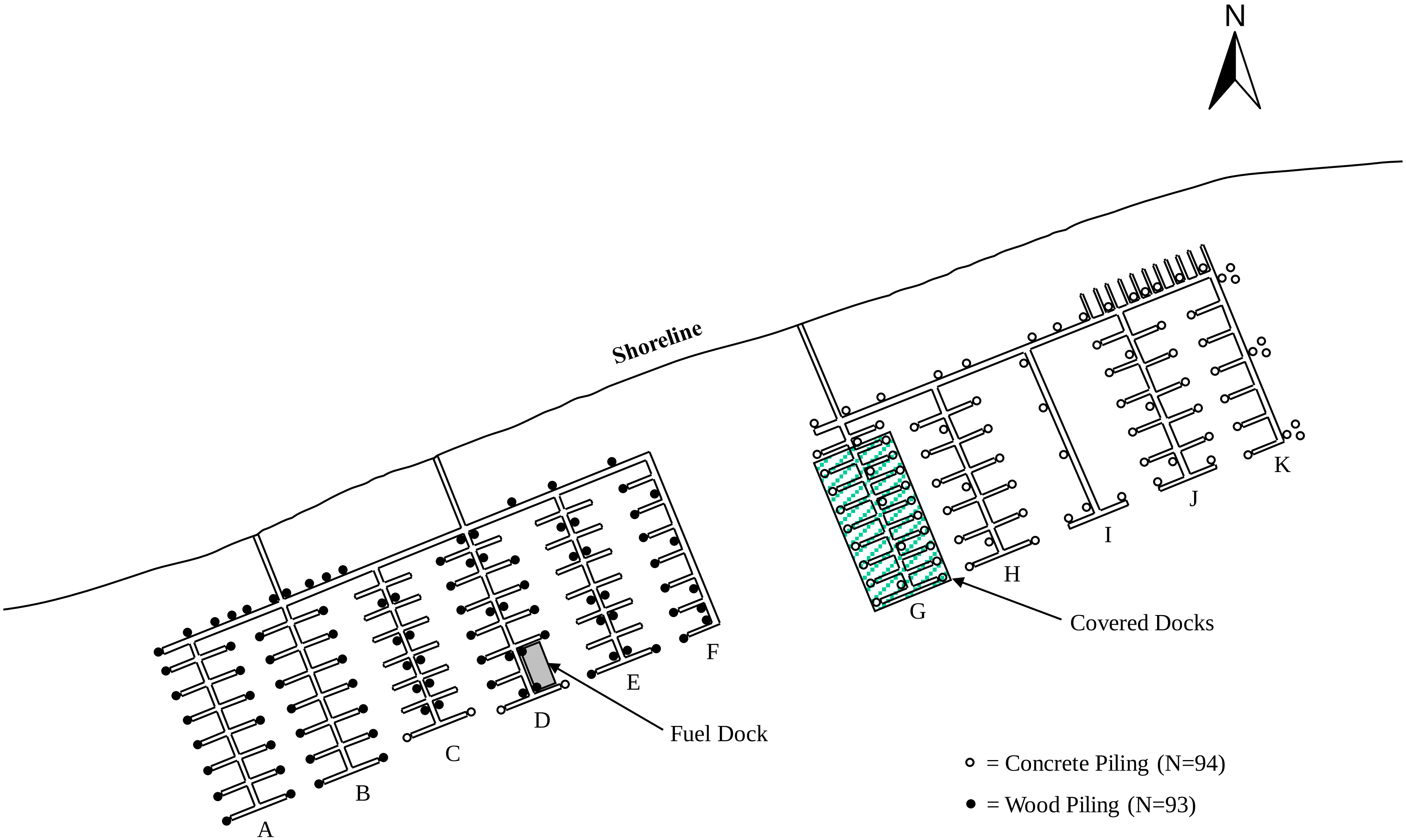
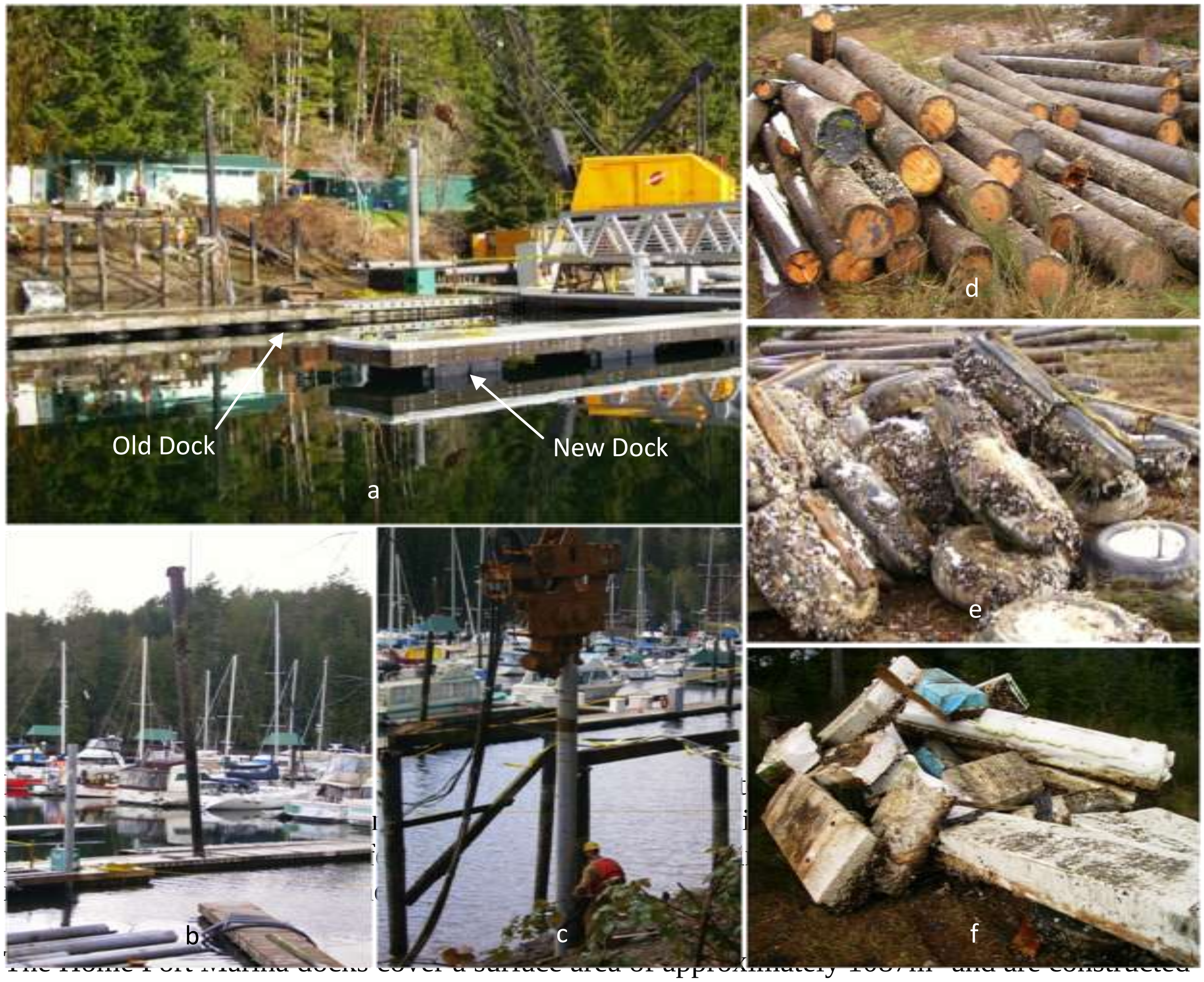


Figure 16. Schematic diagram of Pleasant Harbor Marina drawn to approximate scale.



The Home Port Marina docks cover a surface area of approximately 1087 m^2 and are constructed of foam-filled tires with wood deck surfaces held in place by 53 wood pilings. There is no covered moorage (Figure 18).

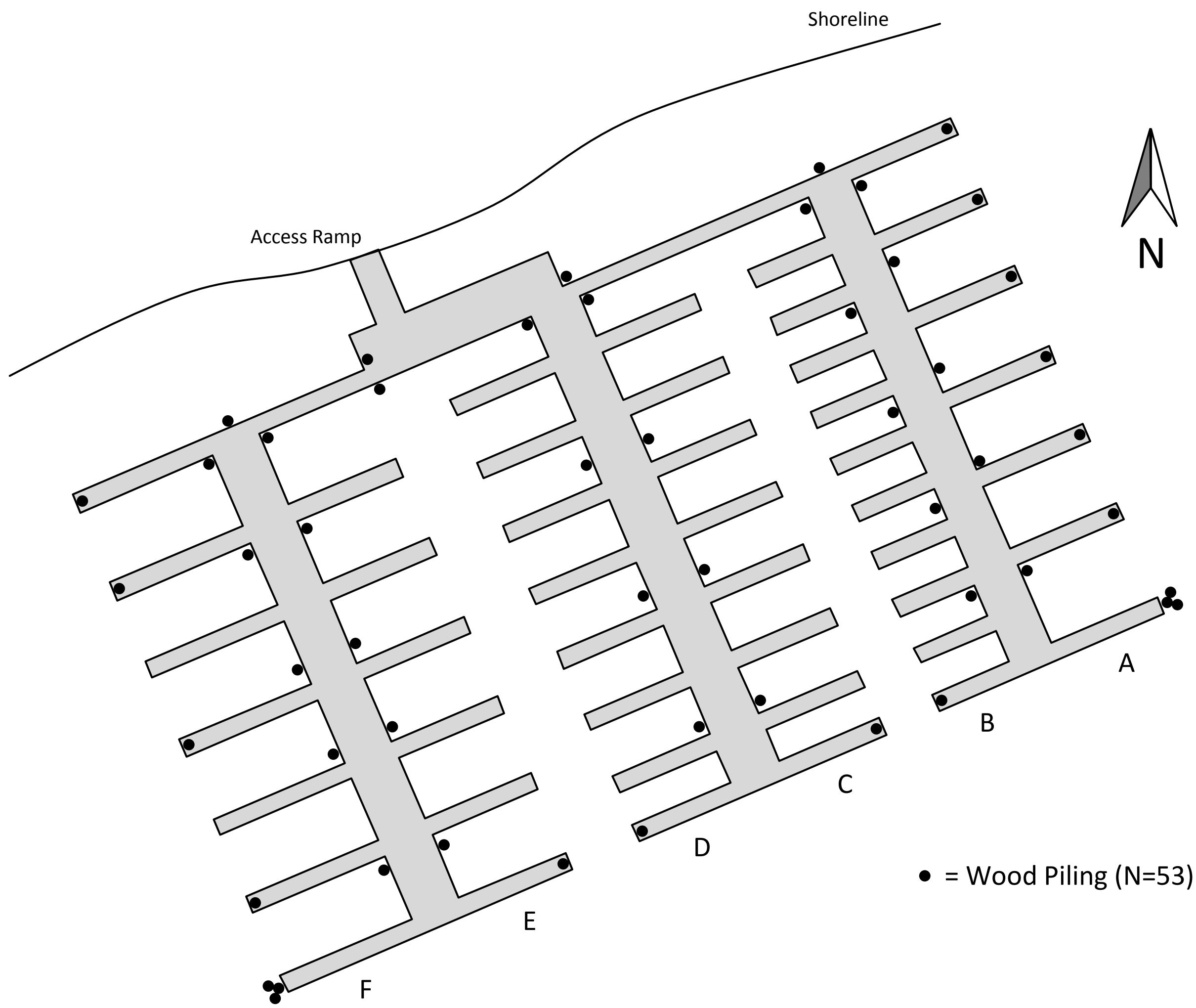
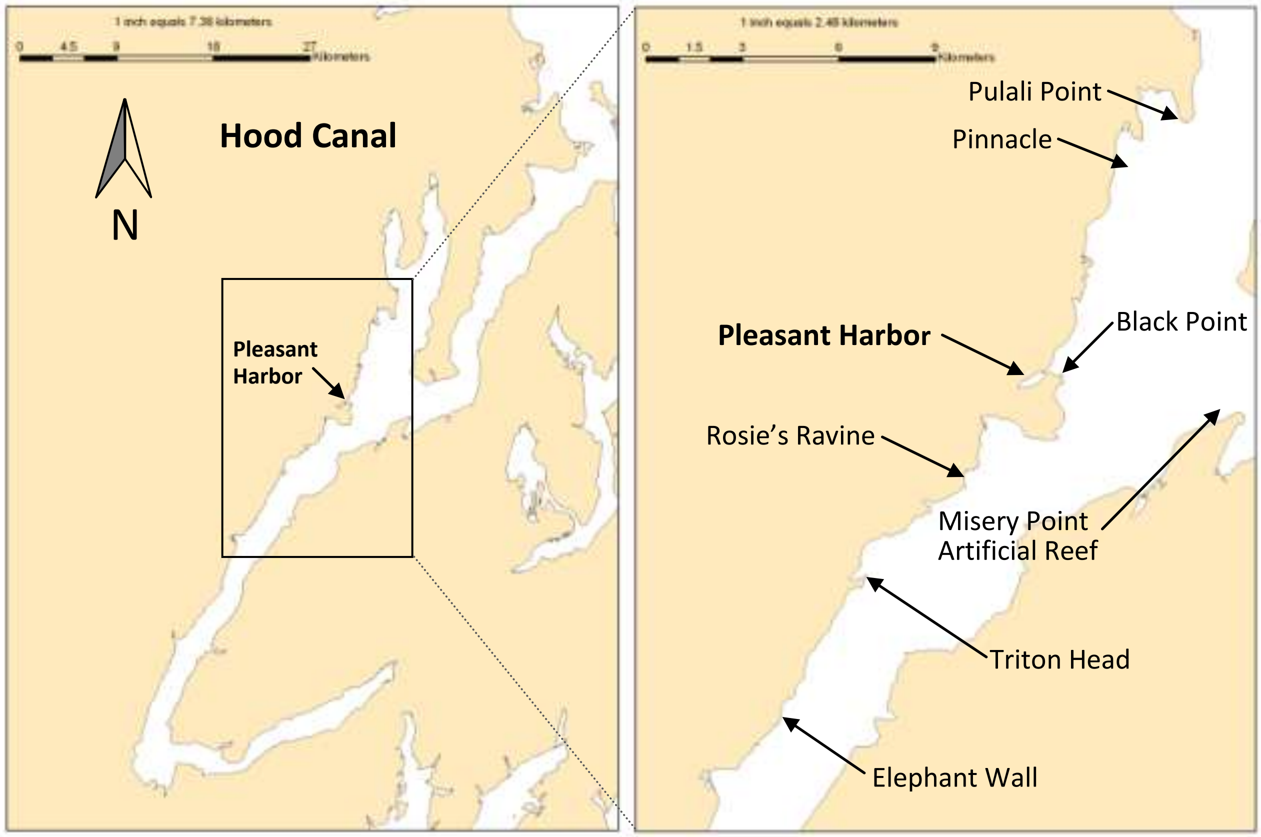


Figure 18. Schematic diagram of Home Port Marina drawn to approximate scale.

In order to evaluate the geographic distribution of *S. clava* both inside and outside the confines of Pleasant Harbor, all nine private residence docks, the WDFW boat ramp dock, and the Pleasant Harbor State Park dock were examined for the presence of *S. clava*. Additionally, six natural bedrock substrate sites and one artificial reef all located within approximately a 14km radius of Pleasant Harbor (Figure 19) were surveyed by AISU divers using a roving diver survey technique whereby divers swim a zigzag pattern across several isobaths throughout the site and note the presence of any non-native tunicates encountered. The surveys were conducted during the months of March, April, and May, 2008. The state park dock was found to be infested, otherwise, no further *S. clava* were encountered which suggests that the Pleasant Harbor infestation is, at present, highly localized and contained within the confines of the bay.



Because *S. clava* are solitary tunicates and individual animals are readily discernible, AISU biologists were able to produce robust estimates of *S. clava* densities, by count, for each of the two marinas. The marinas were mapped and overlaid with $10m^2$ (approximately) section grids. At Pleasant Harbor Marina, 235 sections contained dock structure from which 30 were randomly selected for macrofauna survey and *S. clava* counts (Figure 20). Forty-six sections at Home Port Marina contained dock structure and ten of those sections were randomly selected for survey (Figure 20). Within each section, two randomly positioned, non-overlapping $1m^2$ quadrats were placed on the underside of the dock and surveyed as described for Dockton Park, except that counts of individual tunicates were recorded rather than rank order estimates of percent coverage as was used for *D. vexillum*. All quadrat surveys were conducted by the same two divers henceforth referred to as observers A and B. The Pleasant Harbor Marina surveys were conducted over the course of five days between March 27 and April 21, 2008. Home Port Marina was surveyed on July 22, 2008. Results of the macrofauna surveys are presented in Tables 7 and 8, respectively.

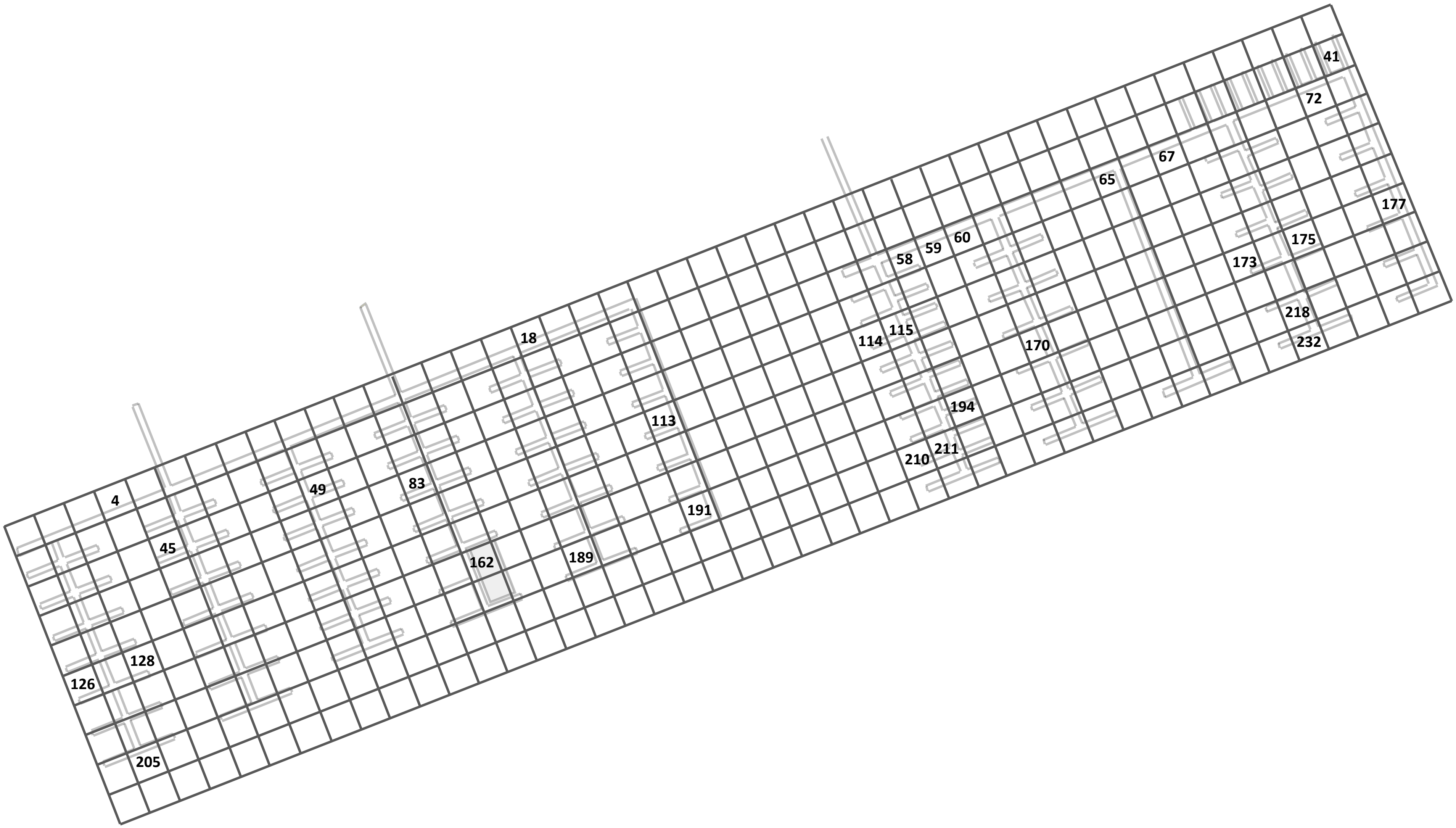


Figure 20. Sample section grid for Pleasant Harbor Marina showing the location of 30 randomly selected sections (out of a total of 235) that were surveyed for macrofauna including *Styela clava* with 1m^2 quadrats. Non-sampled section numbers are omitted for clarity.

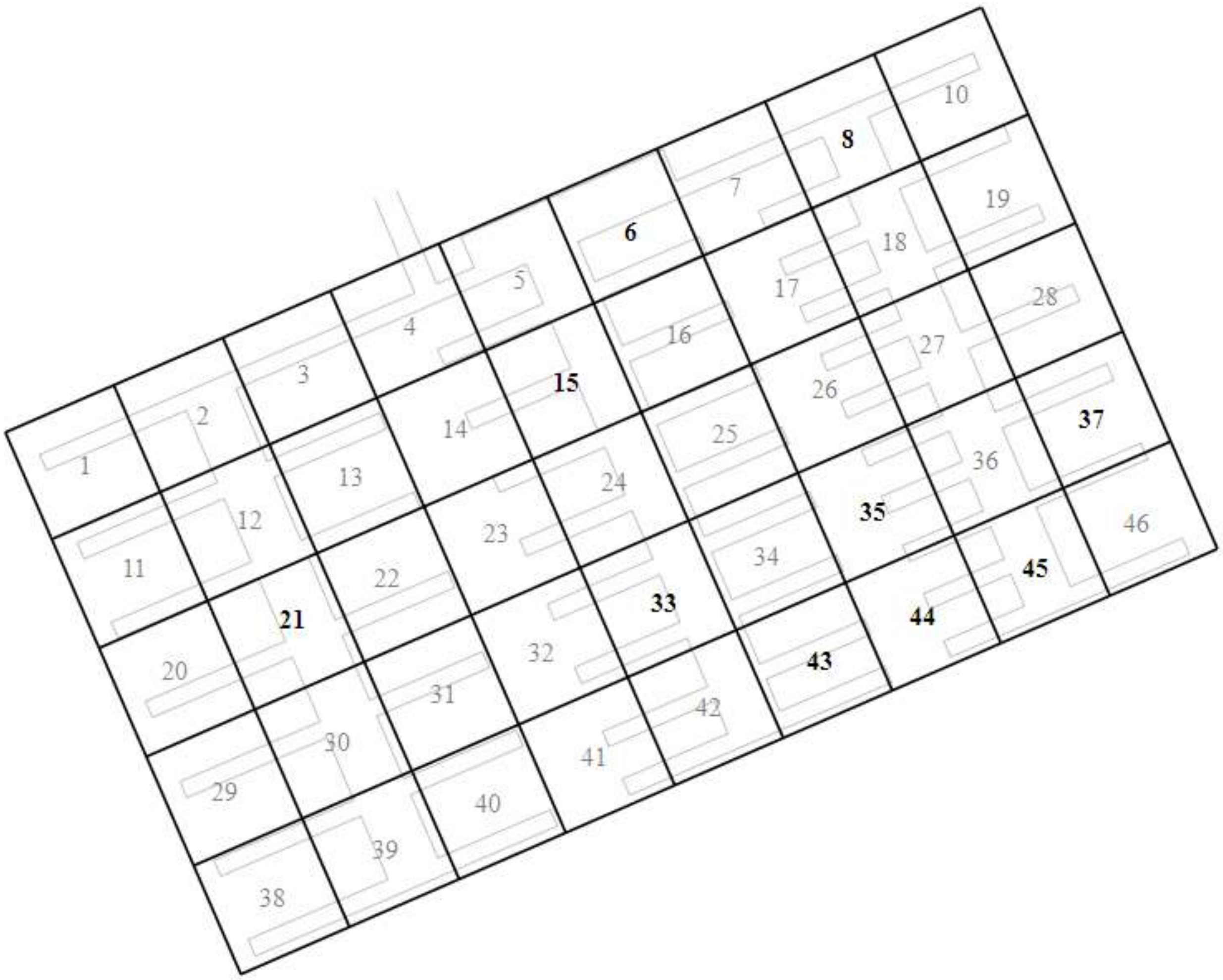


Figure 21. Sample section grid for Home Port Marina showing the location of 10 randomly selected sections (bold) that were surveyed for macrofauna including *Styela clava* with $1m^2$ quadrats.

Table 7. Results of macrofauna presence/absence survey at Pleasant Harbor Marina. *Styela clava* counts represent the mean counts of two independent observers (observers A and B).

Section	4		18		41		45		49		58		59		60		65		67		72		83		113		114		115	
Species	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2
Styela clava (Solitary Tunicate)	4	5	4	4	53	26	16	18	21	13	16	21	14	14	27	14	25	15	14	25	32	16	9	2	6	3	19	23	10	11
Corella inflata (Solitary Tunicate)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Crassostrea gigas (Oyster)	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓	✓	✓	✓
Serpula Sp. (Calcareous Tube Worm)	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Balanus crenatus (Barnacle)		✓		✓	✓	✓	✓	✓		✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Mytilus Sp. (Mussel)			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mopalia Sp. (Chiton)	✓	✓			✓	✓			✓	✓	✓	✓	✓	✓		✓		✓		✓		✓					✓	✓	✓	✓
Sponge Sp. ?				✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pododesmus cepio (Rock Oyster)	✓	✓				✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Metridium senile (Sea anemone)	✓	✓			✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓		✓		✓	✓				✓		✓	✓	✓	✓
Botrylloides violaceus (Colonial Sheath Tunicate)		✓	✓	✓				✓			✓		✓	✓	✓		✓	✓	✓	✓			✓		✓		✓			✓
Botryllus schlosseri (Colonial Star Tunicate)							✓		✓	✓				✓	✓			✓		✓			✓	✓		✓				✓
Strongylocentrotus droebachiensis (Green Sea Urchin)								✓							✓		✓		✓				✓			✓		✓	✓	
Pisaster ochraceus (Ochre Sea Star)			✓		✓	✓						✓		✓							✓	✓	✓						✓	
Hydroid (Obelia sp. ?)				✓	✓										✓		✓		✓		✓						✓			
Hermissenda crassicornis (Opalescent Nudibranch)						✓										✓				✓										✓
Aeolidia papillosa (Shaggy Mouse Nudibranch)																✓			✓											
Didemnum Sp. (Colonial Tunicate)																														
Cnemidocarpa finmarkiensis (Red Solitary Tunicate)					✓												✓													
Eupentacta quinquesemita (White Sea Cucumber)																													✓	
Cucumaria miniata (Orange Sea Cucumber)																														
Parastichopus californicus (California Sea Cucumber)			✓																											
Chlamys hastata (Pink Scallop)		✓						✓							✓		✓					✓					✓		✓	
Pugettia producta (Northern Kelp Crab)											✓																			
Eudistylia vancouveri (Northern Feather Duster worm)																														
Terebratalia transversa (Lampshell)										✓																				

Section	126		128		162		170		173		175		177		189		191		194		205		210		211		218		232	
Species	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2
Styela clava (Solitary Tunicate)	13	12	31	31	17	26	5	7	28	30	32	28	26	8	10	10	0	4	16	17	38	34	40	14	4	17	40	42	23	20
Corella inflata (Solitary Tunicate)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Crassostrea gigas (Oyster)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Serpula Sp. (Calcareous Tube Worm)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Balanus crenatus (Barnacle)	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mytilus Sp. (Mussel)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mopalia Sp. (Chiton)				✓				✓		✓		✓						✓	✓	✓				✓		✓		✓	✓	✓
Sponge Sp. ?	✓	✓		✓	✓	✓		✓	✓	✓		✓		✓	✓	✓		✓		✓	✓	✓	✓			✓	✓	✓		✓
Pododesmus cepio (Rock Oyster)		✓		✓		✓	✓	✓		✓	✓	✓	✓	✓				✓		✓	✓	✓	✓	✓		✓		✓		✓
Metridium senile (Sea anemone)			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Botrylloides violaceus (Colonial Sheath Tunicate)		✓		✓			✓	✓	✓	✓	✓	✓	✓				✓		✓	✓		✓			✓	✓	✓	✓	✓	✓
Botryllus schlosseri (Colonial Star Tunicate)	✓	✓	✓	✓												✓		✓				✓							✓	✓
Strongylocentrotus droebachiensis (Green Sea Urchin)							✓			✓	✓	✓	✓	✓						✓					✓	✓	✓	✓	✓	
Pisaster ochraceus (Ochre Sea Star)				✓						✓		✓	✓	✓			✓		✓											
Hydroid (Obelia sp. ?)						✓	✓	✓	✓		✓		✓																✓	
Hermissenda crassicornis (Opalescent Nudibranch)											✓	✓		✓																✓
Aeolidia papillosa (Shaggy Mouse Nudibranch)												✓		✓																
Didemnum Sp. (Colonial Tunicate)																								✓						
Cnemidocarpa finmarkiensis (Red Solitary Tunicate)										✓																				
Eupentacta quinquesemita (White Sea Cucumber)																				✓			✓		✓	✓				
Cucumaria miniata (Orange Sea Cucumber)																				✓						✓				
Parastichopus californicus (California Sea Cucumber)																														
Chlamys hastata (Pink Scallop)									✓		✓								✓	✓						✓		✓		
Pugettia producta (Northern Kelp Crab)																														
Eudistylia vancouveri (Northern Feather Duster worm)		✓																												
Terebratalia transversa (Lampshell)																														

Table 8. Results of macrofauna presence/absence **s**urvey at Home Port Marina. *Styela clava* counts represent the mean counts of two independent observers (observers A and B).

	Section																			
	6		8		15		21		33		35		37		43		44		45	
Species	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2
Styela clava (Solitary Tunicate)	12	4	0	2	8	4	2	2	3	7	1	1	0	0	4	0	1	0	1	1
Crassostrea gigas (Oyster)							✓								✓	✓				
Serpula Sp. (Calcareous Tube Worm)		✓		✓		✓		✓		✓		✓				✓		✓		✓
Balanus Sp. (Barnacle)	✓	✓	✓	✓			✓	✓		✓	✓	✓		✓	✓	✓				✓
Mytilus Sp. (Mussel)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sponge Sp. ?		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pododesmus cepio (Rock Oyster)													✓			✓				
Metridium senile (Sea anemone)								✓						✓		✓		✓		
Botrylloides violaceus (Colonial Sheath Tunicate)	✓		✓			✓	✓	✓	✓	✓	✓						✓		✓	✓
Botryllus schlosseri (Colonial Star Tunicate)		✓		✓	✓				✓	✓	✓	✓		✓	✓	✓	✓		✓	✓
Pisaster ochraceus (Ochre Sea Star)			✓				✓						✓							
Hydroids *Obelia Sp.* ?	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓
Eudistylia vancouveri (Northern Feather Duster worm)			✓										✓							

To ensure that reliable density estimates by count could be achieved using multiple observers, tests for variability in counts among observers were conducted. The divers traded positions at the conclusion of their respective macrofauna survey and, without sharing counts with the other observer, conducted another count of *S. clava* in the second quadrat (Table 9). A paired sample t test and Pearson correlation coefficient was used to test for similarity in counts. The counts for either marina did not differ significantly ($p>0.05$), and the correlation coefficients were high (0.79 and 0.89 for Pleasant Harbor Marina and Home Port Marina, respectively). Reasoning that the likelihood of an observer failing to count individual tunicates that were present but not noticed was greater than counting individual tunicates that were not present, overall densities were computed by taking the mean summed over all quadrats using the greater of the two counts for any single quadrat when the counts differed. This approach produces a less conservative density estimate than using the mean from the two observer's counts. The computed *S. clava* densities for Pleasant Harbor and Home Port Marinas were $20/m^2$ and $3/m^2$ respectively. Quantitative macrofauna surveys and *S. clava* density estimates were not produced for the Pleasant Harbor State Park dock.

Table 9. *Styela clava* counts made by two independent observers at Pleasant Harbor and Home Port Marinas. Numbers in bold denote counts that were used to produce the overall estimates of *S. clava* densities at each of the two marinas.

Pleasant Harbor Marina

Section #	Quadrat #	Observer A	Observer B	Section #	Quadrat #	Observer A	Observer B
4	1	2	**6**	126	1	12	**13**
	2	4	**6**		2	**13**	10
18	1	2	**6**	128	1	**35**	26
	2	2	**6**		2	**41**	21
41	1	51	**54**	162	1	12	**21**
	2	**28**	24		2	23	**29**
45	1	**20**	12	170	1	4	**6**
	2	17	**19**		2	7	**7**
49	1	19	**23**	173	1	**28**	27
	2	10	**15**		2	**30**	29
58	1	**18**	14	175	1	**32**	31
	2	**24**	18		2	21	**35**
59	1	11	**17**	177	1	20	**31**
	2	14	**14**		2	6	**9**
60	1	23	**30**	189	1	**10**	10
	2	**16**	12		2	**10**	9
65	1	23	**27**	191	1	0	**0**
	2	**17**	13		2	**4**	4
67	1	**15**	12	194	1	**19**	12
	2	**26**	23		2	**17**	16
72	1	29	**35**	205	1	31	**41**
	2	**16**	16		2	33	**34**
83	1	8	**10**	210	1	31	**48**
	2	**3**	1		2	9	**18**
113	1	3	**9**	211	1	2	**5**
	2	**4**	2		2	15	**19**
114	1	**20**	18	218	1	40	**40**
	2	23	**23**		2	**48**	36
115	1	9	**11**	232	1	21	**24**
	2	**11**	11		2	19	**21**

Home Port Marina

Section #	Quadrat #	Observer A	Observer B	Section #	Quadrat #	Observer A	Observer B
6	1	10	**13**	35	1	0	**2**
	2	3	**5**		2	**1**	0
8	1	0	**0**	37	1	0	**0**
	2	1	**2**		2	**0**	0
15	1	4	**11**	43	1	3	**4**
	2	**4**	4		2	0	**0**
21	1	**2**	1	44	1	0	**2**
	2	2	**2**		2	**0**	0
33	1	**3**	3	45	1	0	**1**
	2	6	**7**		2	**1**	0

Des Moines Marina

Des Moines Marina is located on the east shore of the Main Basin of Puget Sound and lies within the Central Puget Sound Management Region (see Plates 1 & 4). It is owned and operated by the city of Des Moines. Its docks are constructed of a mixture of concrete and polystyrene pontoons held in place by wood and concrete pilings (Figure 22). The non-native tunicate *Ciona savignyi* was first reported at Des Moines in 1998.

Quantitative surveys of the docks were conducted in order to produce a robust density estimate of *C. savignyi* for the marina. The facility was mapped and overlaid with a 10 m^2 (approximately) section grid and surveyed as previously described for Pleasant Harbor. One hundred sixteen sections contained dock structure from which forty sections were randomly selected for macrofauna survey and *C. savignyi* counts (Figure 23). Duplicate blind counts of *C. savignyi* were conducted by the same two divers (observers A and B) used at Pleasant Harbor to assess variability in counts among divers for this species. The counts did not differ significantly ($p>0.05$) between the two observers, and the correlation coefficient (0.79) was high (Table 10). The surveys were conducted over the course of seven days between June 19, and July 15, 2008. Results of the macrofauna presence/absence survey are presented in Table 11. Overall density was computed by taking the mean summed over all 80 quadrats using the greater of the two counts for any single quadrat. The density of *C. savignyi* at Des Moines Marina was 116/m^2.

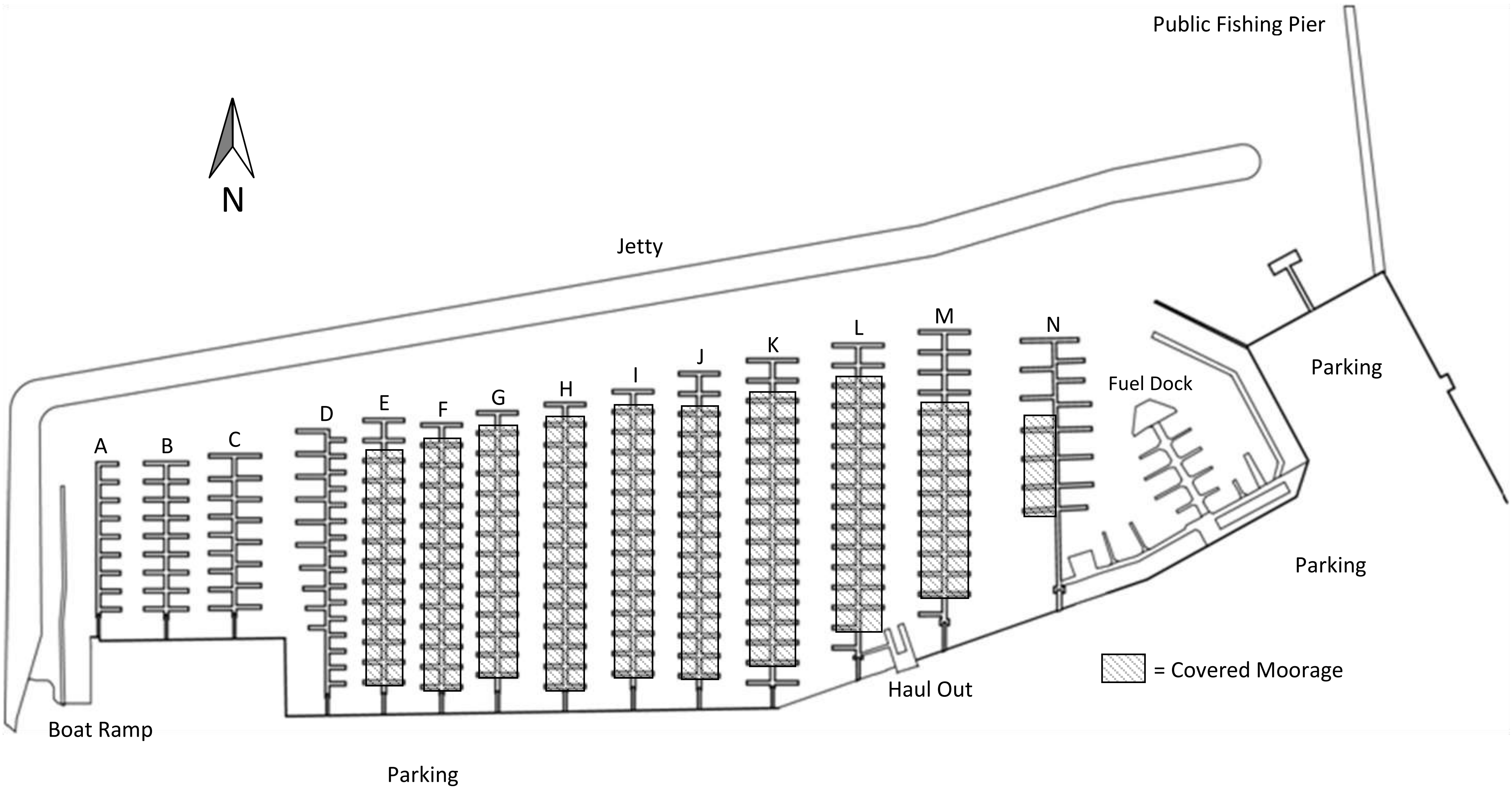


Figure 22. Schematic diagram of Des Moines Marina drawn to approximate scale.

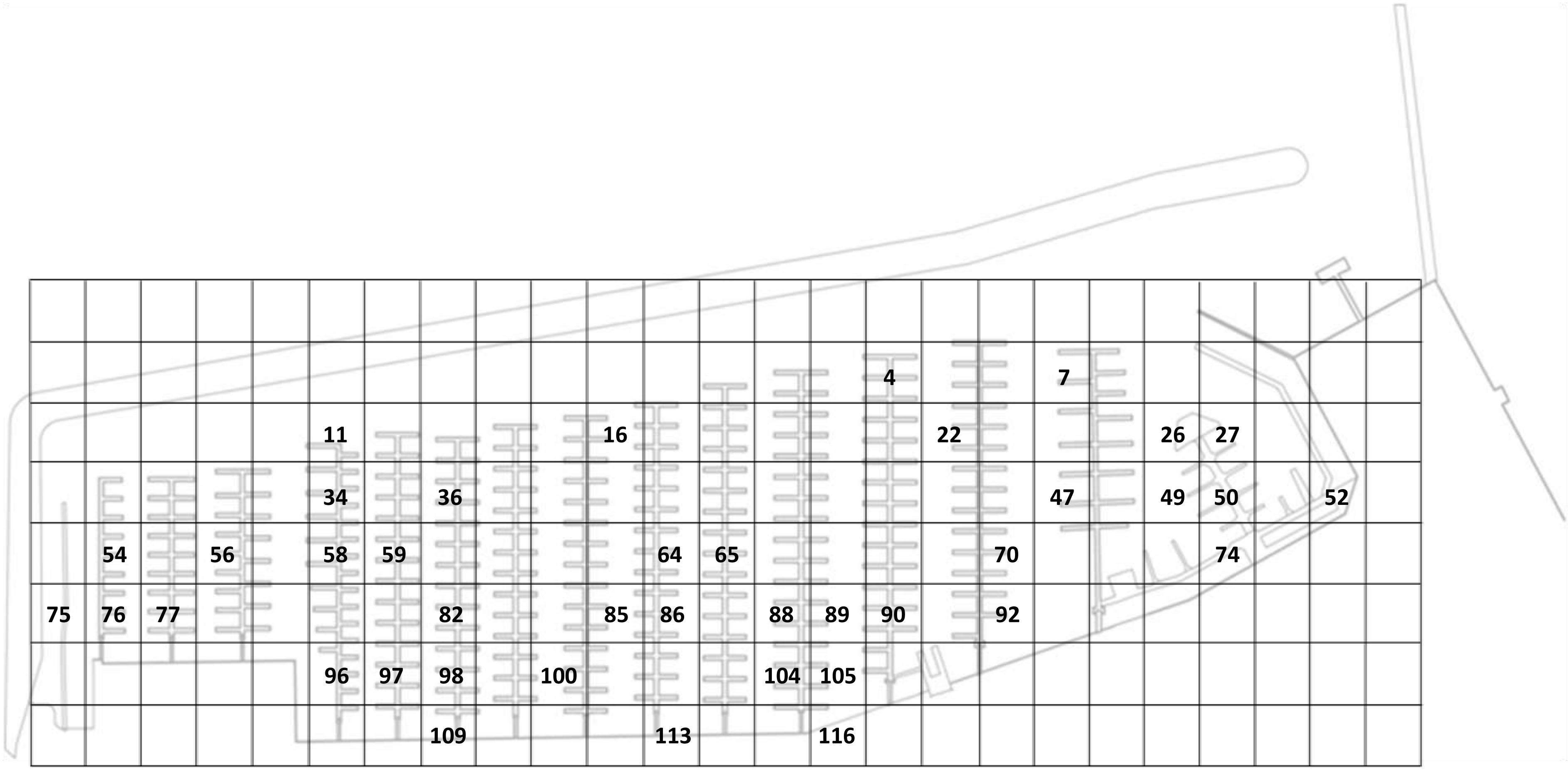


Figure 23. Sample section grid showing the location of 40 randomly selected sections that were surveyed for macrofauna including *Ciona savignyi* with 1 m^2 quadrats at Des Moines Marina. Non-sampled section numbers are omitted for clarity.

Table 10. *Ciona savignyi* counts made by two independent observers at Des Moines Marina. Numbers in bold denote counts that were used to produce the overall estimate of *C. savignyi* density.

Des Moines Marina							
Section #	Quadrat #	Observer A	Observer B	Section #	Quadrat #	Observer A	Observer B
4	1	32	**51**	74	1	122	**140**
	2	43	**60**		2	**117**	90
7	1	13	**28**	75	1	49	**75**
	2	24	**26**		2	**117**	70
11	1	39	**53**	76	1	**83**	82
	2	42	**50**		2	54	**57**
16	1	**81**	77	77	1	**129**	110
	2	63	**65**		2	**119**	90
22	1	57	**77**	82	1	156	**167**
	2	**67**	51		2	**118**	95
26	1	8	**23**	85	1	125	**162**
	2	23	**37**		2	89	**98**
27	1	11	**27**	86	1	80	**93**
	2	26	**30**		2	100	**130**
34	1	65	**86**	88	1	**120**	113
	2	83	**128**		2	**128**	127
36	1	82	**93**	89	1	139	**181**
	2	97	**100**		2	108	**135**
47	1	**64**	52	90	1	**88**	63
	2	**122**	98		2	**178**	115
49	1	63	**87**	92	1	**121**	98
	2	**211**	115		2	**114**	90
50	1	133	**198**	96	1	112	**137**
	2	**146**	141		2	139	**178**
52	1	114	**130**	97	1	166	**176**
	2	**157**	107		2	**197**	184
54	1	50	**64**	98	1	100	**101**
	2	102	**105**		2	111	**115**
56	1	109	**119**	100	1	**71**	69
	2	138	**141**		2	**158**	103
58	1	**107**	107	104	1	183	**195**
	2	132	**172**		2	118	**143**
59	1	80	**80**	105	1	107	**121**
	2	136	**142**		2	152	**153**
64	1	97	**120**	109	1	139	**149**
	2	107	**115**		2	86	**106**
65	1	81	**85**	113	1	157	**250**
	2	**108**	80		2	166	**255**
70	1	**101**	83	116	1	**203**	157
	2	**141**	89		2	**138**	98

Table 11. Results of macrofauna presence/absence survey at Des Moines Marina. *Ciona savignyi* counts represent the mean counts of two independent observers (observers A and B).

Section

Species	4		7		11		16		22		26		27		34		36		47		49		50		52		54		56		58		59		64		65		70	
	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2
Ciona savignyi (Solitary Tunicate)	42	52	21	25	46	46	79	64	67	58	16	38	19	28	76	106	88	99	58	110	75	183	166	144	122	132	57	104	114	140	107	152	80	130	106	111	83	94	92	115
Sponge Sp. ?		✓		✓		✓	✓	✓		✓		✓		✓		✓		✓	✓	✓		✓		✓		✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓
Botryllus schlosseri (Colonial Star Tunicate)		✓						✓	✓	✓					✓		✓			✓		✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Botrylloides violaceus (Colonial Sheath Tunicate)	✓	✓	✓	✓	✓	✓	✓	✓		✓			✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓				✓	
Corella inflata (Solitary Tunicate)						✓		✓								✓		✓														✓						✓		
Balanus crenatus (Barnacle)				✓		✓			✓	✓		✓		✓						✓		✓			✓	✓						✓					✓	✓		✓
Mytilus Sp. (Mussel)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓					✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pododesmus cepio (Rock Oyster)	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
Serpula Sp. (Calcareous Tube Worm)	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Eudistylia vancouveri (Northern Feather Duster worm)	✓		✓		✓		✓		✓		✓		✓			✓		✓					✓			✓	✓										✓			✓
Crassostrea gigas (Oyster)											✓																													
Mopalia Sp. (Chiton)												✓		✓								✓				✓	✓	✓												
Metridium senile (Sea anemone)		✓		✓	✓	✓		✓	✓	✓	✓	✓		✓		✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓		✓		✓		✓	✓	✓	✓	✓	✓	✓
Strongylocentrotus droebachiensis (Green Sea Urchin)								✓													✓									✓										
Pisaster ochraceus (Ochre Sea Star)	✓											✓	✓															✓												
Cucumaria piperata (Speckled Sea Cucumber)																							✓																	
Eupentacta quinquesemita (White Sea Cucumber)	✓	✓			✓	✓									✓			✓			✓	✓	✓	✓	✓		✓		✓	✓	✓	✓								
Cucumaria miniata (Orange Sea Cucumber)																							✓						✓	✓										✓
Hermissenda crassicornis (Opalescent Nudibranch)	✓			✓		✓				✓																		✓				✓		✓						
Dirona albolineata (White lined Dirona)																																								
Hydroid (*Obelia* Sp. ?)	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Styela gibsii (Solitary Tunicate)		✓				✓	✓	✓		✓		✓		✓	✓	✓	✓	✓	✓	✓		✓		✓		✓		✓	✓	✓	✓	✓		✓		✓		✓	✓	
Didemnum Sp. (Colonial Tunicate)											✓							✓			✓				✓															
Pugettia producta (Northern Kelp Crab)																																								
Myxicola infundibulum (Slime Tubeworm)	✓				✓	✓									✓	✓					✓		✓				✓		✓	✓	✓			✓						
Chlamys hastata (Pink Scallop)																																								

Species	74		75		76		77		82		85		86		88		89		90		92		96		97		98		100		104		105		109		113		116	
	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2	Quadrat 1	Quadrat 2
Ciona savignyi (Solitary Tunicate)	131	184	62	94	83	56	120	105	162	187	144	94	87	115	117	128	160	122	76	147	110	182	125	156	171	191	101	113	70	131	189	131	114	153	144	96	204	211	180	118
Sponge Sp. ?		✓		✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓		✓		✓		✓	✓	✓		✓
Botryllus schlosseri (Colonial Star Tunicate)	✓	✓		✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓		✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓
Botrylloides violaceus (Colonial Sheath Tunicate)	✓			✓	✓	✓		✓	✓	✓		✓				✓	✓			✓	✓		✓	✓		✓	✓		✓				✓				✓		✓	✓
Corella inflata (Solitary Tunicate)								✓		✓		✓				✓								✓				✓		✓		✓						✓		✓
Balanus crenatus (Barnacle)				✓		✓					✓		✓	✓				✓	✓	✓		✓								✓		✓	✓	✓						
Mytilus Sp. (Mussel)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pododesmus cepio (Rock Oyster)	✓	✓		✓	✓	✓	✓	✓		✓		✓		✓	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓		✓		✓		✓	✓	✓		✓		✓	✓	
Serpula Sp. (Calcareous Tube Worm)	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓
Eudistylia vancouveri (Northern Feather Duster worm)	✓	✓	✓	✓	✓		✓										✓					✓	✓	✓									✓							
Crassostrea gigas (Oyster)																																								
Mopalia Sp. (Chiton)	✓	✓																						✓																
Metridium senile (Sea anemone)	✓	✓	✓	✓			✓	✓		✓		✓		✓		✓		✓		✓				✓		✓	✓	✓		✓	✓	✓		✓		✓		✓		✓
Strongylocentrotus droebachiensis (Green Sea Urchin)								✓																✓																✓
Pisaster ochraceus (Ochre Sea Star)			✓	✓		✓																✓										✓								
Cucumaria piperata (Speckled Sea Cucumber)						✓																																		
Eupentacta quinquesemita (White Sea Cucumber)			✓		✓		✓	✓						✓									✓	✓				✓					✓							
Cucumaria miniata (Orange Sea Cucumber)								✓																																
Hermissenda crassicornis (Opalescent Nudibranch)							✓														✓		✓									✓								
Dirona albolineata (White lined Dirona)																				✓																				
Hydroid (*Obelia* Sp. ?)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Styela gibsii (Solitary Tunicate)		✓		✓		✓	✓	✓	✓	✓		✓		✓		✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Didemnum Sp. (Colonial Tunicate)		✓	✓	✓																																				
Pugettia producta (Northern Kelp Crab)																											✓		✓											
Myxicola infundibulum (Slime Tubeworm)					✓		✓	✓															✓										✓							
Chlamys hastata (Pink Scallop)			✓		✓												✓						✓	✓														✓		

Port Angeles Boat Haven Marina

Port Angeles is located in the western Strait of Juan de Fuca management region. It is a major point of entry for recreational and commercial vessels arriving from outside the U.S., and the city supports a broad diversity of maritime industries. It is the first harbor capable of accommodating large ships of commerce on the eastward approach to Puget Sound from the Pacific Ocean and it is a popular layover for recreational vessels entering or exiting Washington's inland marine waters. Due to its gateway location to Puget Sound and the high number of vessels trafficking through the port, the AISU considers Port Angeles to be of particular concern as a potential point of entry for non-native tunicates and at high risk for infestation. A cursory exam of a few of the docks using SCUBA and drop-camera video failed to detect the presence of non-native tunicates. However, because the facility is considered high risk, AISU biologists elected to conduct a more thorough assessment of the docks.

The facility was mapped and overlaid with a $10m^2$ (approximately) section grid and surveyed as previously described for other marinas (Figure 24). Three hundred thirty-eight sections contained dock structure from which fifty sections were randomly selected for macrofauna survey (Figure 25). The native solitary tunicate *Corella inflata* was among the dominant macro-invertebrates found on the dock undersides. Though quantitative estimates of abundance were not produced for *C. inflata*, they were present in what appeared to be moderate to high densities in nearly every section surveyed. Some scattered small colonies of *D. vexillum* were noted; however, neither of the other two non-native tunicate species of greatest concern (*S. clava* and *C. savignyi*) was observed (Table 12). In addition to the section surveys, paired divers swam zigzag patterns along several sections of the main arterial docks. Observations from those surveys were consistent with results from the section surveys and the initial SCUBA/drop camera inspection. The surveys were conducted over the course of five days: August 27 and 28, and September 2, 3, and 23, 2008.

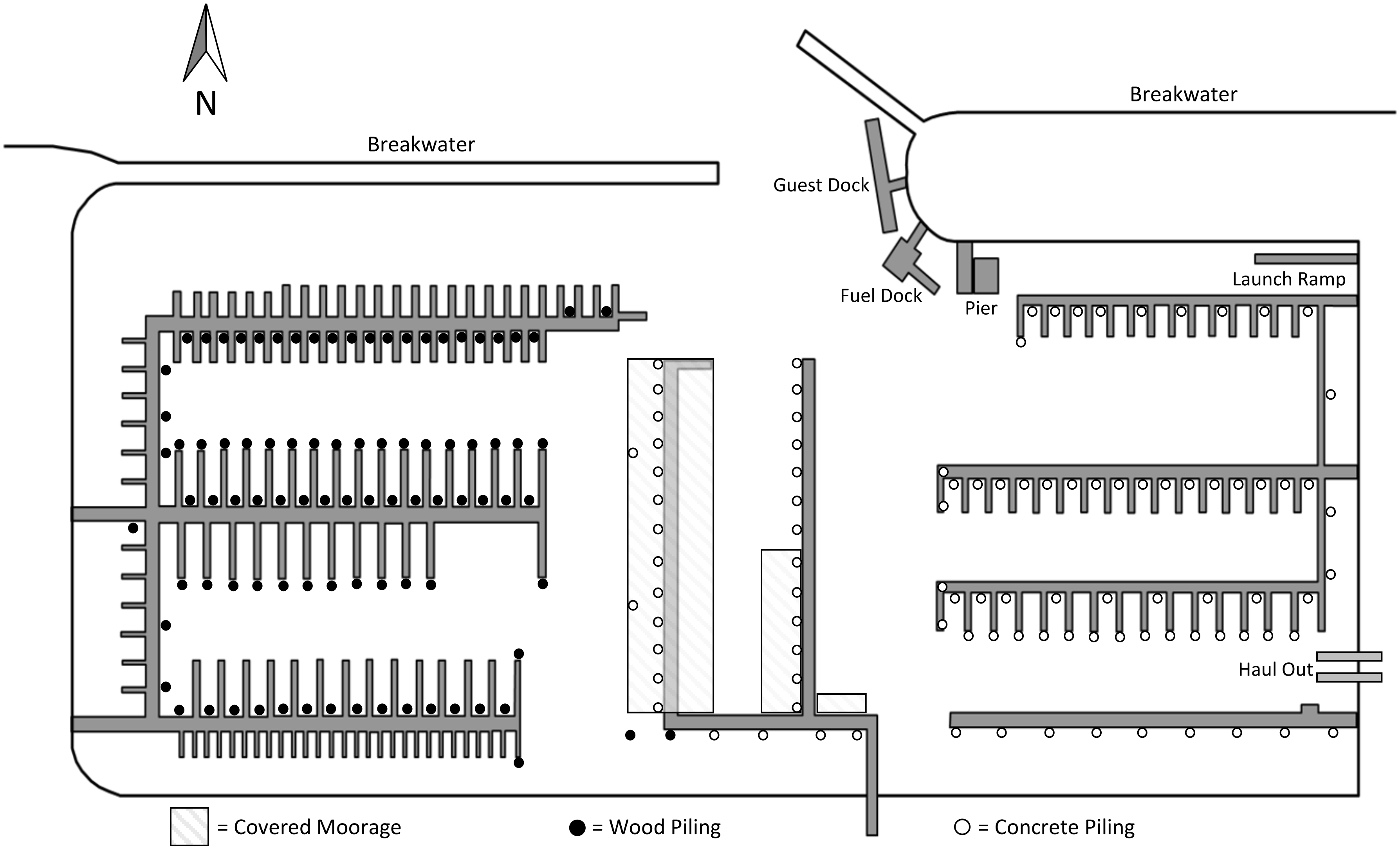


Figure 24. Schematic diagram of Port Angeles Boat Haven Marina drawn to approximate scale.

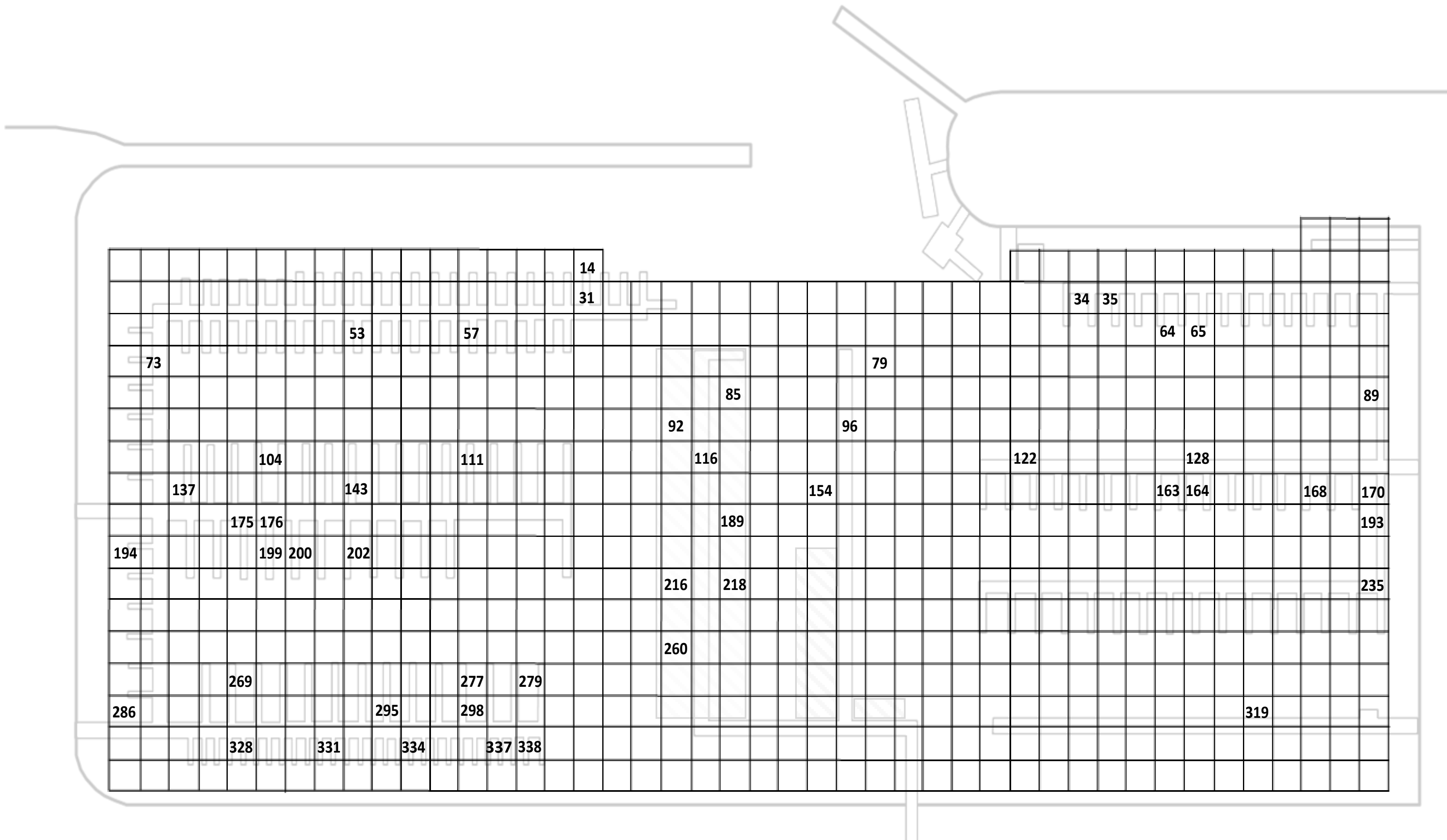


Figure 25. Sample section grid showing the location of 50 randomly selected sections that were surveyed for macrofauna with 1 m^2 quadrats at Port Angeles Boat Haven Marina. Non-sampled section numbers are omitted for clarity.

Table 12. Results of macrofauna presence/absence survey at Port Angeles Boat Haven Marina.

Species	14		31		34		35		53		57		64		65		73		79		85		88		92		96		104		111		116		122		128		137		143		154		163		164		168		170		175		176		189		193		194		199		200		202		216		218		235		256		259		277		279		286		295		298		319		328		331		334		337		338	
Ciona intestinalis (Solitary Tunicate)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓		✓	✓	✓
Serpula Sp. (Calcareous Tube Worm)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓			✓								✓	✓		✓								✓	✓	✓	✓											✓	✓	✓	✓	✓					✓	✓						✓	✓	✓		✓	✓	✓			✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓		✓	✓	✓	✓	✓	✓
Balanus crenatus (Barnacle)		✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓		✓			✓		✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓			✓	✓			✓	✓	✓	✓		✓		✓	✓	✓			✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓		✓
Mytilus Sp. (Mussel)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓	✓			✓		✓	✓	✓				✓	✓	✓	✓			✓	✓		✓	✓	✓	✓	✓	✓	✓					✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓		✓			✓	✓	✓	✓	✓	✓				✓	✓	✓	✓	✓		✓		✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mopalia Sp. (Chiton)		✓															✓	✓				✓																																																													✓	✓			✓									✓		✓	✓	
Encrusting Sponge Sp. 1	✓	✓		✓		✓	✓	✓	✓	✓	✓	✓						✓				✓		✓		✓											✓	✓		✓		✓								✓		✓	✓		✓			✓											✓	✓		✓			✓	✓		✓	✓	✓	✓	✓		✓			✓	✓				✓		✓			✓			✓
Ribbon Sponge Sp. 1																					✓				✓				✓												✓																✓				✓						✓		✓		✓				✓		✓						✓		✓						✓	✓	✓							
Pododesmus cepio (Rock Oyster)																																																																																					✓	✓								✓								
Metridium senile (Sea anemone)	✓	✓	✓	✓					✓	✓	✓	✓					✓	✓			✓	✓				✓			✓			✓				✓				✓	✓	✓	✓	✓						✓				✓		✓		✓				✓							✓	✓	✓	✓			✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Botrylloides violaceus (Colonial Sheath Tunicate)	✓	✓	✓	✓	✓		✓		✓	✓	✓	✓					✓	✓	✓	✓	✓	✓	✓		✓	✓		✓	✓	✓	✓	✓				✓			✓		✓	✓		✓									✓		✓		✓	✓			✓		✓					✓		✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓							✓	✓	✓		✓	
Botryllus schlosseri (Colonial Star Tunicate)						✓		✓						✓		✓								✓						✓														✓						✓		✓		✓								✓		✓	✓																													
Styela gibsii (Solitary Tunicate)																																				✓														✓							✓	✓																		✓														✓												
Styela montereyensis (Solitary Tunicate)																																																									✓																								✓													✓				✓
Strongylocentrotus droebachiensis (Green Sea Urchin)																																																																																						✓									✓				
Hydroid Obelia Sp. ?	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓			✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓			✓			✓		✓			✓	
Bryozoans						✓		✓						✓																																✓						✓																						✓																										
Hermissenda crassicornis (Opalescent Nudibranch)					✓																															✓													✓	✓																																																		
Dirona albolineata (White lined Dirona)	✓																																													✓														✓																						✓																		
Didemnum Sp. (Colonial Tunicate)															✓								✓																												✓																																																
Cnemidocarpa finmarkiensis (Red Solitary Tunicate)						✓																																																																				✓																										
Eupentacta quinquesemita (White Sea Cucumber)																																																																																																✓				
Chlamys hastata (Pink Scallop)																																																																																						✓																	
Pugettia producta (Northern Kelp Crab)																						✓				✓																																✓																✓				✓								✓														
Oregonia gracilis (Decorator Crab)																																																																								✓				✓																									
Eudistylia vancouveri (Northern Feather Duster worm)				✓						✓												✓			✓																		✓	✓															✓	✓			✓												✓	✓			✓	✓						✓		✓				✓			✓	✓	✓	✓	✓	✓	✓	✓		✓
Myxicola infundibulum (Slime Tubeworm)				✓													✓	✓																																✓							✓	✓			✓	✓													✓	✓		✓	✓		✓	✓	✓	✓	✓	✓	✓				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Elliot Bay Marina

Elliot Bay Marina is a privately owned marina located on the north shore of Elliot Bay in Puget Sound's Main Basin. The docks are constructed of concrete pontoons and are held in place by concrete pilings (Figure 26). *Ciona savignyi* was first reported present at the marina in 2007. Additionally in 2007, a single confirmed *S. clava* was found attached to the underside of a dock. No *S. clava* have since been found at this location.

Owing to the marina's large size, its layout along a cardinal compass axis, and its unobstructed shoreline, the facility was divided into four large sections, each containing approximately equal surface area to test for density differences by proximity to shore and by direction. Each of the four sections was further subdivided into 10 m^2 (approximately) sections. Within each of the four sections, ten randomly selected 10 m^2 sections were surveyed for macrofauna, including *C. savignyi*, as previously described for other locations (Figure 27). Results from the survey are presented in Table 13. A third diver (observer C) replaced observer A, thus providing a further test of among diver variability in counts for this species. The counts did not differ significantly ($p>0.05$) between the two observers, and the correlation coefficient (0.94) was high (Table 14). The 20 sample sections from the two large nearshore (northern) sections were combined (40 quadrats total) and compared by paired sample t test to the 40 offshore (southern) quadrats. The result was not significant ($P>0.05$). However, when the two eastern sections were combined and compared to the two western sections combined, the result was highly significant ($P<0.001$). The mean density from the eastern half of the marina was more than twice that of the western half (132 m^2 and 62 m^2, respectively). The densities used to test for spatial differences within the marina were computed using the means of the two observers summed over all quadrats within each of the two halves. The overall density was computed as described for other locations- by taking the mean summed over all 80 quadrats using the greater of the two counts for any single quadrat. The overall density of *C. savignyi* at Elliot Bay Marina was 103/m^2, only slightly less than the density recorded for Des Moines Marina.

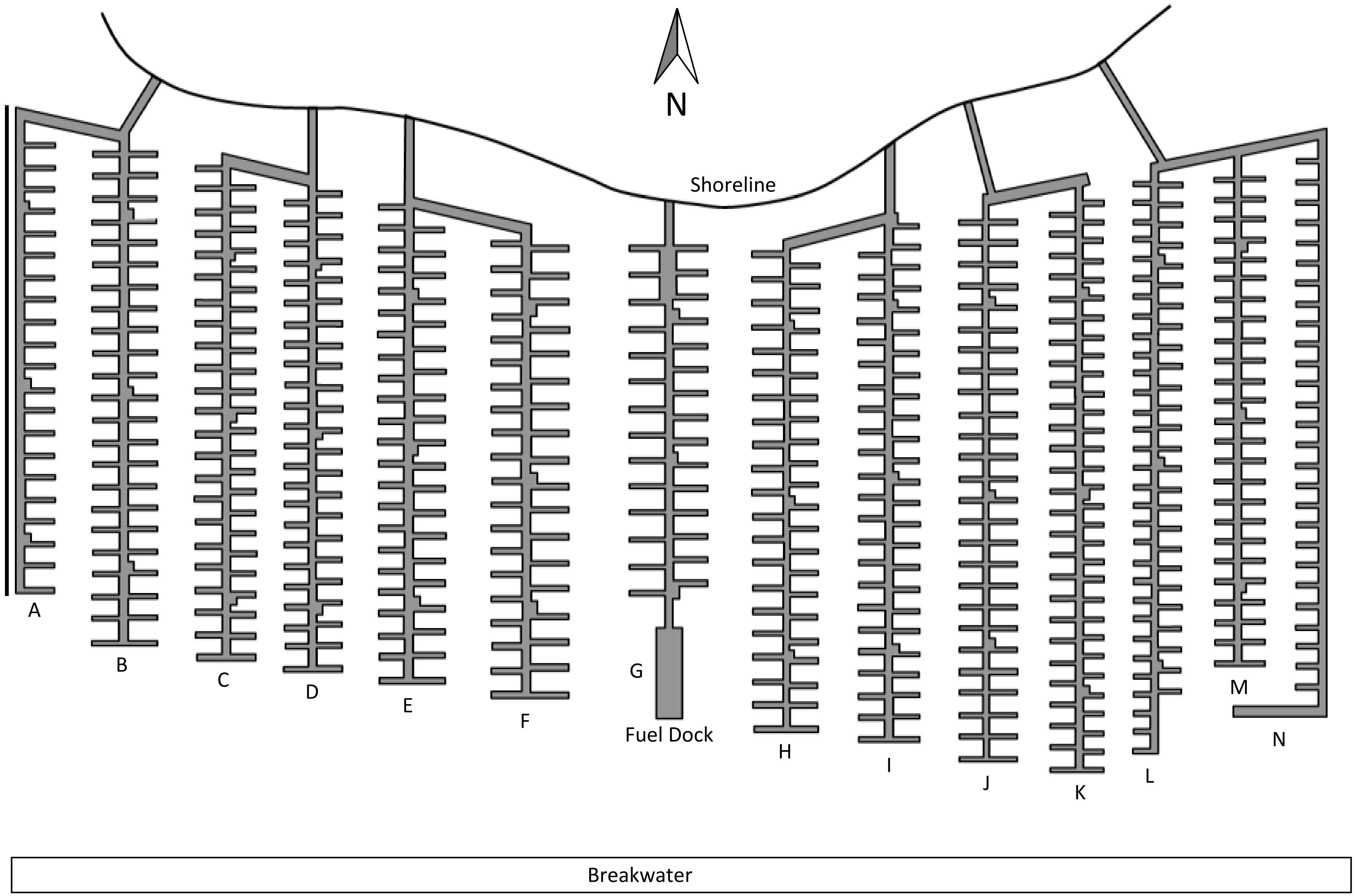


Figure 26. Schematic diagram of Elliot Bay Marina drawn to approximate scale.

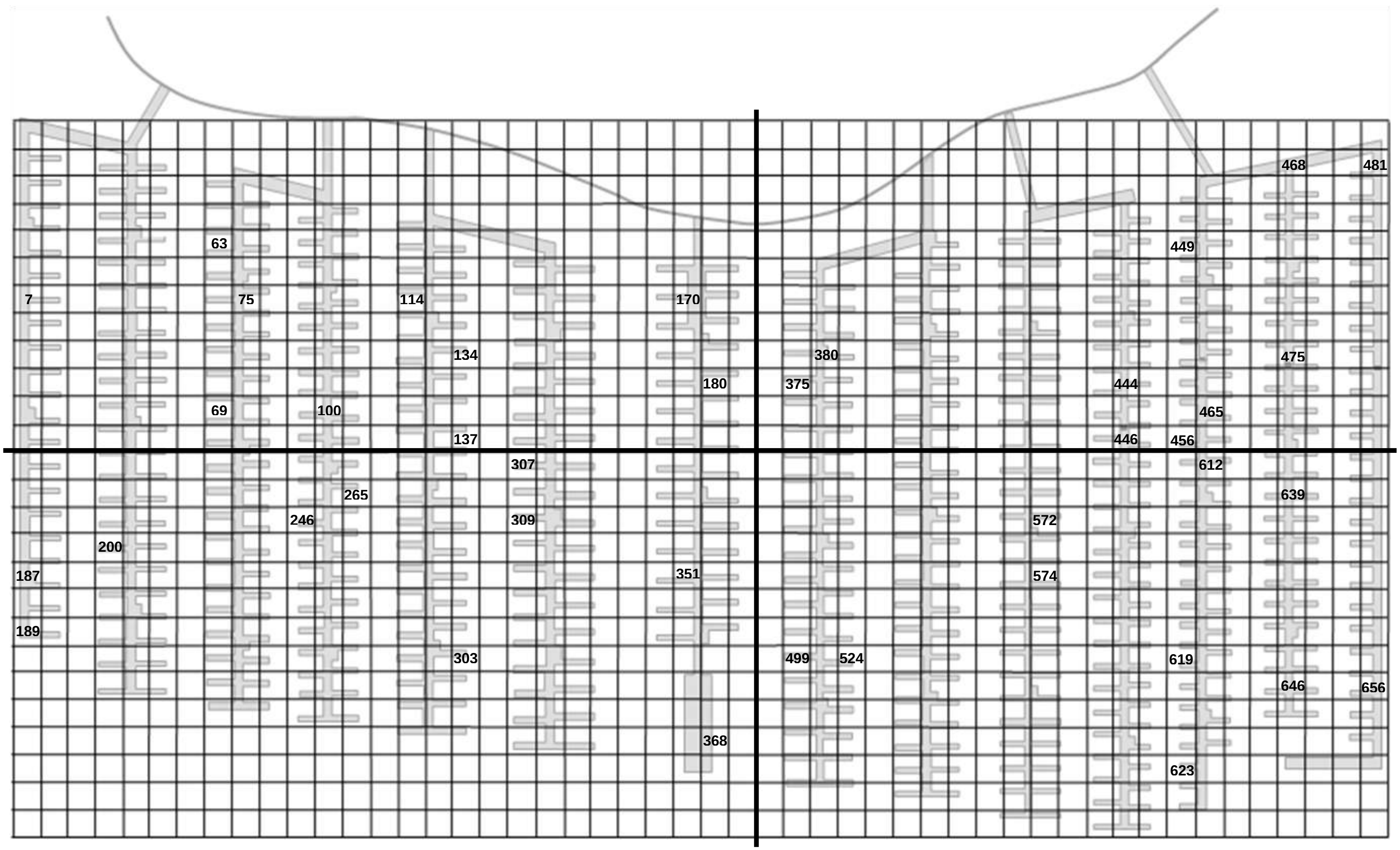


Figure 27. Sample section grid showing the location of 10 randomly selected 10 m^2 sections within each of four larger sections that were surveyed for macrofauna including *Ciona savignyi* with 1 m^2 quadrats. Non-sampled section numbers are omitted for clarity.

Table 13. Results of macrofauna presence/absence survey at Elliot Bay Marina. *Ciona savignyi* counts represent the mean counts of two independent observers (observers B and C).

Species	7		63		69		75		100		114		134		137		170		180		187		189		200		246		265		303		307		309		351		368		375		380		444		446		449		456		465		468		475		481		499		524		572		574		612		619		623		639		646		656	
	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2	Quadrant 1	Quadrant 2
Ciona savignyi (Solitary Tunicate)	58	45	89	80	128	149	129	175	184	145	240	172	171	161	164	153	88	82	87	124	126	114	61	84	87	132	165	107	179	189	210	159	152	186	173	196	117	108	65	75	148	127	73	92	45	37	89	73	20	8	46	54	61	33	48	37	36	40	18	28	96	101	105	144	67	76	129	99	88	32	78	79	37	43	24	24	38	42	12	18
Sponge Sp. ?	✓	✓	✓		✓		✓		✓		✓		✓		✓		✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓		✓	✓	✓		✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓
Botryllus schlosseri (Colonial Star Tunicate)	✓	✓	✓	✓	✓		✓		✓		✓	✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓	✓		✓				✓	✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓
Botrylloides violaceus (Colonial Sheath Tunicate)	✓		✓				✓						✓			✓			✓													✓											✓		✓						✓		✓				✓				✓						✓								✓					
Corella inflata (Solitary Tunicate)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓					✓		✓
Molgula manhattensis (Sea Grape Tunicate)	✓		✓	✓	✓		✓		✓				✓		✓				✓		✓				✓		✓				✓		✓		✓								✓		✓	✓					✓	✓	✓		✓	✓	✓		✓		✓		✓		✓				✓		✓		✓		✓	✓	✓	✓		
Cnemidocarpa finmarkiensis (Shiny Tunicate)																																													✓				✓						✓	✓			✓	✓									✓								✓			
Boltenia villosa or *Pyura haustor* (Solitary Tunicates)	✓	✓		✓	✓		✓		✓	✓					✓		✓	✓	✓	✓		✓		✓	✓	✓	✓				✓													✓	✓	✓	✓		✓	✓	✓	✓	✓	✓									✓			✓	✓	✓		✓										
Balanus crenatus (Barnacle)	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓				✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓			✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓	✓	✓		✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓	✓	✓
Mytilus Sp. (Mussel)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Pododesmus cepio (Rock Oyster)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓
Serpula Sp. (Calcareous Tube Worm)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Eudistylia vancouveri (Northern Feather Duster worm)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Crassostrea gigas (Oyster)																																																																												✓				
Mopalia Sp. (Chiton)																	✓																				✓					✓	✓						✓						✓	✓	✓	✓	✓		✓											✓	✓					✓		✓
Metridium senile (Sea anemone)		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓	✓	✓		✓	✓	✓		✓		✓				✓	✓	✓		✓	✓	✓		✓		✓	✓	✓	✓	✓	✓			✓	✓	✓
Strongylocentrotus droebachiensis (Green Sea Urchin)		✓															✓						✓															✓		✓					✓	✓					✓		✓				✓												✓			✓		✓						
Pycnopodia helianthoides (Sunflower Sea Star)																																																					✓																											
Pisaster ochraceus (Ochre Sea Star)																																																								✓																								
Cucumaria piperata (Speckled Sea Cucumber)																		✓																																																														
Eupentacta quinquesemita (White Sea Cucumber)	✓	✓		✓	✓	✓	✓	✓	✓			✓	✓	✓	✓					✓	✓		✓		✓		✓		✓	✓			✓	✓			✓			✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓		✓			✓		✓	✓	✓		✓	✓					✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		
Cucumaria miniata (Orange Sea Cucumber)		✓												✓	✓	✓						✓				✓								✓								✓								✓				✓	✓	✓	✓	✓																	✓	✓				✓
Hermissenda crassicornis (Opalescent Nudibranch)																						✓																				✓								✓			✓																			✓								
Hydroid (Obelia Sp. ?)	✓	✓	✓		✓	✓	✓	✓	✓		✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓		✓	✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓
Styela gibbsii (Solitary Tunicate)																																																							✓																									
Myxicola infundibulum (Slime Tubeworm)	✓	✓		✓		✓		✓		✓						✓		✓		✓		✓		✓			✓	✓				✓		✓		✓								✓		✓		✓		✓		✓		✓		✓				✓		✓		✓		✓		✓				✓		✓		✓				
Chlamys hastata (Pink Scallop)		✓																																✓																																														

Table 14. *Ciona savignyi* counts made by two independent observers at Elliot Bay Marina. Numbers in bold denote counts that were used to produce the overall estimate of *C. savignyi* density.

Elliot Bay Marina							
Section #	Quadrat #	Observer B	Observer C	Section #	Quadrat #	Observer B	Observer C
7	1	**69**	47	375	1	143	**153**
	2	**49**	41		2	100	**154**
63	1	87	**91**	380	1	66	**79**
	2	77	**82**		2	91	**92**
69	1	**137**	121	444	1	43	**46**
	2	**161**	136		2	**37**	36
75	1	124	**134**	446	1	65	**72**
	2	**176**	173		2	53	**93**
100	1	159	**209**	449	1	20	**20**
	2	143	**146**		2	**8**	7
114	1	**262**	218	456	1	**48**	44
	2	**172**	171		2	52	**55**
134	1	159	**182**	465	1	59	**62**
	2	**163**	158		2	30	**36**
137	1	154	**174**	468	1	46	**50**
	2	145	**161**		2	31	**43**
170	1	91	**84**	475	1	**41**	31
	2	60	**64**		2	**41**	39
180	1	**92**	81	481	1	15	**21**
	2	121	**127**		2	25	**30**
187	1	124	**127**	499	1	**99**	99
	2	**115**	112		2	161	**161**
189	1	**63**	59	524	1	94	**116**
	2	71	**96**		2	**149**	139
200	1	**108**	86	572	1	88	**105**
	2	**133**	130		2	65	**86**
246	1	160	**169**	574	1	120	**137**
	2	86	**127**		2	**101**	96
265	1	174	**184**	612	1	**74**	61
	2	158	**179**		2	28	**35**
303	1	**221**	198	619	1	77	**79**
	2	**176**	142		2	**80**	78
307	1	**167**	137	623	1	**37**	37
	2	150	**228**		2	40	**46**
309	1	**179**	167	639	1	**27**	21
	2	**195**	184		2	21	**26**
351	1	**137**	96	646	1	**37**	35
	2	**125**	91		2	**48**	36
368	1	60	**69**	656	1	12	**12**
	2	68	**81**		2	14	**21**

Non-native Tunicate Removal From Vessel Hulls

In an attempt to limit the spread of non-native tunicates from heavily infested marinas via vessel traffic, WDFW divers and various dive companies under contract to the AISU, have conducted hand removals from vessel hulls. Annual removals began in 2006 at those marinas known to be infested with *S. clava* (Pleasant Harbor, Home Port, Blaine, and Semiahmoo Marinas) and annual removals of *C. savignyi* from Des Moines and Elliot Bay Marinas commenced in 2007. Results are summarized in Table 15.

Pleasant Harbor

In May and June of 2006, the AISU contracted with the Skokomish Tribe and with Global Dive and Salvage, Inc. to survey vessel hulls and to remove, by hand, *Styela clava* from those vessels found to be infested at both Pleasant Harbor and Home Port Marinas, and from vessel hulls moored to private residence docks within the confines of Pleasant Harbor. During March and April of 2007, the survey and removal was conducted by WDFW Marine Resources Division divers. Biologists from the AISU completed the survey and removal during April and May 2008 and again in June 2009.

Des Moines Marina

In June, 2007, the AISU contracted with Seattle Diving Co. to remove, by hand, *C. savignyi* from the hulls of all vessels present at the marina during that time. Seventeen vessels were so severely infested that the divers determined that hand removal was not practical. These vessels did not appear to have left their moorings for an extended period of time and were designated extensions of the dock. Of the remaining vessels, 44 were infested and 598 individual *C. savignyi* were removed. The total number of vessels surveyed was not recorded. During May and March, 2008 and March and April 2009, the AISU contracted with Ballard Diving and Salvage, Inc. to conduct the hand removal. As in 2007, a small number of vessels were heavily infested and appeared not to have moved over an extended period, though in 2008 and 2009, efforts were made to remove all *C. savignyi* from their hulls.

On May 27, 2008, as a quality control measure, AISU divers identified 25 infested vessels prior to the contract diver's hand removal effort. On June 5, subsequent to the hand removal, the vessels were re-inspected and 11 remained infested. The contractor was notified and, upon review of their records, found that the same diver was assigned to nearly all 11 vessels. The contractor agreed to remove tunicates from all 25 vessels and further agreed to re-inspect and remove any additional tunicates from all other vessels assigned to that diver.

Elliot Bay Marina

In June, 2007, the AISU contracted with Seattle Diving Co. to remove, by hand, *C. savignyi* from the hulls of all vessels present at the marina during that time. In June, 2008 and April, 2009, Ballard Diving and Salvage, Inc. was contracted to conduct the removals.

Blaine and Semihamoo Marinas

The AISU contracted with Natural Resource Consultants to conduct removals of *S. clava* from vessel hulls in Blaine and Semihamoo Marinas during May, 2006. In April and May 2007, Seattle Diving Co. was contracted to conduct the removals, and in June 2008 and April 2009, the contract was awarded to Ballard Diving and Salvage, Inc..

Table 15. Summary of non-native tunicate removals from vessel hulls.

Location/Species removed	Month(s), Year	# vessels surveyed	# vessels infested	# non-native tunicates removed	# non-native tunicates removed/# vessels surveyed	Contract Vendor/Agency
Pleasant Harbor Marina/*Styela clava*	May-June, 2006	169	24	917	5.4	Skokomish Tribe/Global Dive and Salvage, Inc.
	March-April, 2007	124	39	1,420	11.5	Washington Department of Fish and Wildlife
	April-May, 2008	168	48	803	4.8	Washington Department of Fish and Wildlife
	May, 2009	165	42	381	2.3	Washington Department of Fish and Wildlife
Home Port Marina/ *Styela clava*	June, 2006	72	12	533	7.4	Skokomish Tribe/Global Dive and Salvage, Inc.
	April-May, 2007	52	10	97	1.9	Washington Department of Fish and Wildlife
	July, 2008	74	14	178	2.4	Washington Department of Fish and Wildlife
	May, 2009	65	10	55	0.8	Washington Department of Fish and Wildlife
Plesant Harbor Private Residence Docks/ *Styela clava*	June, 2006	13	4	0[1]	NA	Skokomish Tribe/Global Dive and Salvage, Inc.
	April-May, 2007	15	6	259	17.3	Washington Department of Fish and Wildlife
	July, 2008	10	1	2	0.2	Washington Department of Fish and Wildlife
	May, 2009	8	1	3	0.4	Washington Department of Fish and Wildlife
Des Moines Marina/ *Ciona savignyi*	June, 2007	NA[2]	44	598	NA	Seattle Diving Co.
	May, 2008	696	107	4,134	5.9	Ballard Diving and Salvage, Inc.
	March-April, 2009	642	142	4,043	6.3	Ballard Diving and Salvage, Inc.
Elliot Bay Marina/ *Ciona savignyi*	June, 2007	NA[2]	65	1,529	NA	Seattle Diving Co.
	June, 2008	1,029	141	5,291	5.1	Ballard Diving and Salvage, Inc.
	April, 2009	1,015	245	26,889	26.5	Ballard Diving and Salvage, Inc.
Blaine Marina/ *Styela clava*	May, 2006	521	93	3,545	6.8	Natural Resources Consultants
	April-May, 2007	505	87	2,329	4.6	Seattle Diving Co.
	June, 2008	507	26	816	1.6	Ballard Diving and Salvage, Inc.
	April, 2009	533	13	41	0.08	Ballard Diving and Salvage, Inc.
Semiahmoo Marina/ *Styela clava*	May, 2006	211	17	82	0.4	Natural Resources Consultants
	May, 2007	211	19	281	1.3	Seattle Diving Co.
	June, 2008	195	0	0	0.0	Ballard Diving and Salvage, Inc.
	April, 2009	204	1	1	0.0	Ballard Diving and Salvage, Inc.

[1] The surveyors did not acquire permission from vessel owners to remove *Styela clava*.
[2] The number of vessels surveyed was not recorded.

Response to the Trans-Pacific Relocation of a Large Semi-Submersible Barge

In December, 2008, the AISU learned of the transport of a large semi-submersible barge arriving from Zhanjiaj, China, approximately 480 Km west of Hong Kong. The barge was built in Japan in 1969 and later refitted and moved to Zhanjiaj where it had served as a transport barge since 2002. It was purchased by a company operating a shipyard in Bellingham Bay and in November, 2008, it was towed across the Pacific with brief fueling stops in Pusun, Korea and Dutch Harbor, Alaska. The vessel arrived at the Port of Bellingham Shipping Terminal Pier on January 7, 2009 and was briefly moved to a shipyard pier on the south shore of Bellingham Bay before being moved back to the Port of Bellingham Shipping Terminal Pier. Divers from the AISU arrived to inspect the vessel for non-native species on January 8; however, high winds, rough surface conditions, and extremely limited visibility precluded inspection. Divers returned on February 7 and were able to conduct a thorough investigation that included the full length and breadth of the vessel and its seachests (Figure 28). The hull was nearly completely covered with calcareous plate remains of the barnacle *Megabalanus coccopoma*; however, no living barnacles were found.

Figure 28. *Lucky Angel* (*Faithful Servant*) in Bellingham Bay.

Megabalanus coccopoma is native to the tropical and sub-tropical eastern Pacific and has, in recent years, been identified as an invasive species along the eastern seaboard and Gulf coast states of the U.S.. It has also been reported from the western Indian Ocean, Asia, and parts of Europe. The barnacle remains found on the hull were largely intact and still exhibited the rosy coloration characteristic for this species, which suggests that the barnacles may have been living when the vessel was taken in tow. The crossing route took the vessel through the temperate and sub-arctic water regimes of the north Pacific and any barnacles that were living on the hull in China were likely killed in transit due to low cold water tolerance. No other non-native fouling organisms were found on the hull.

Dewatto Rock Wall, Hood Canal

During a fish habitat survey in the fall of 2008, biologists from the WDFW Marine Fish Program reported an abundance of *C. savignyi* along a deep-water rock wall near the mouth of Dewatto Bay in southern Hood Canal. On December 11, 2009, AISU biologists deployed an underwater remotely operated vehicle (ROV) to conduct a qualitative survey for *C. savignyi* along the full length of the wall, and to evaluate the ROV for its potential use in acquiring quantitative data on *C. savignyi* density and geographic distribution in deep-water habitat (Figure 29). *Ciona savignyi* was found to be abundant and nearly uniformly distributed along the full length (nearly 1nm) and depth (up to 12m) of the wall. The ROV proved to be a valuable tool for deep-water observations and showed great promise for collecting quantitative assessment data on tunicate distribution and abundance.

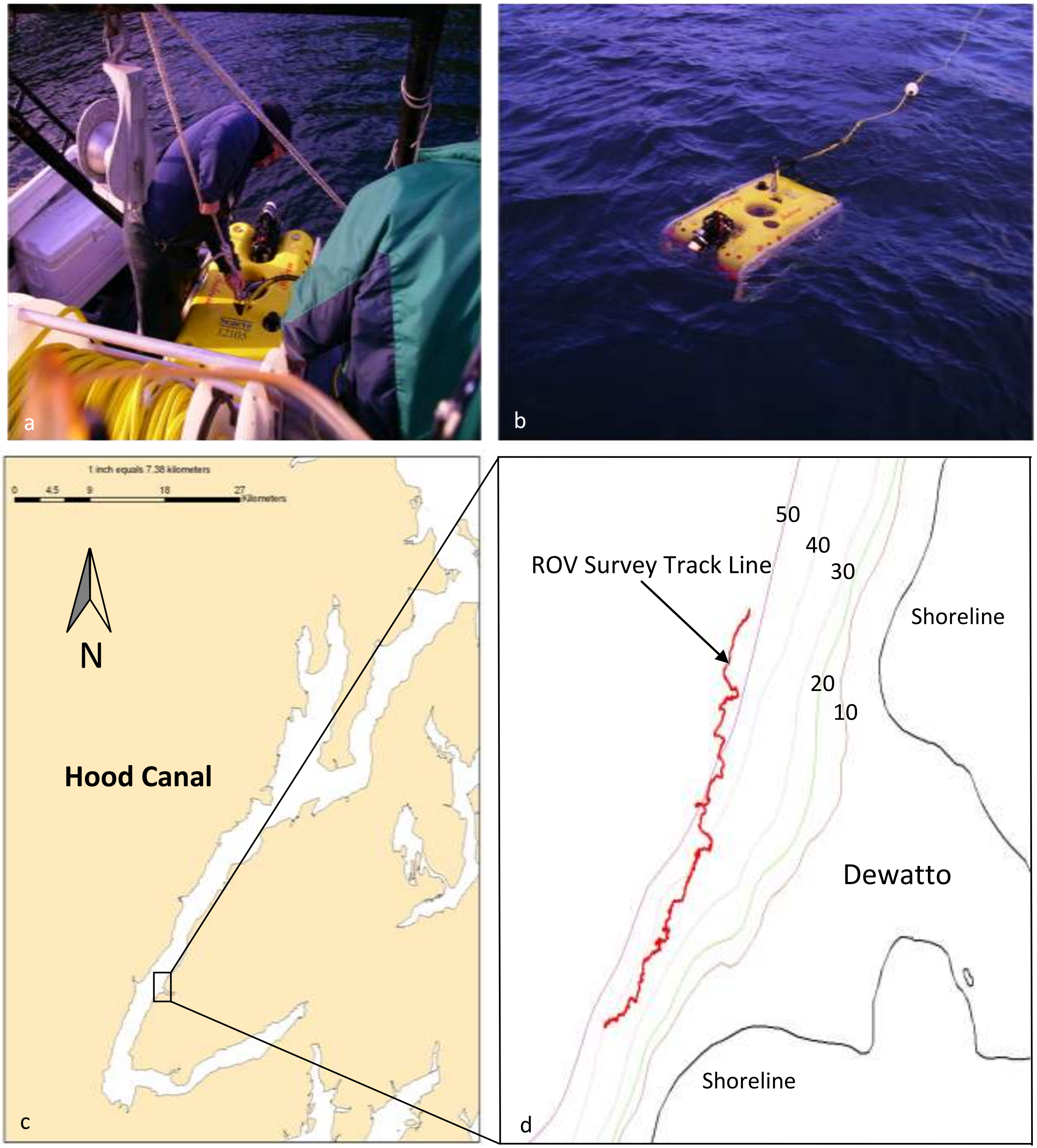


Figure 29. a and b) Deployment of remotely operated vehicle (ROV), c) Location of Dewatto Bay, d) ROV track line used for qualitative survey of *Ciona savignyi* along the rock wall near Dewatto Bay. Bathymetries are in fathoms.

Conclusions and Recommendations for 2009 Biennium

The AISU has completed each of the tasks outlined in the contract with the PSP. In addition to the information presented in this report, the AISU has completed and circulated a working draft of the statewide Tunicate Management Plan, participated with, and provided annual review to, the TRAC, and advised the PSP on their "Keep Your Hull Clean" outreach campaign. Relevant information is being posted on the WDFW

Survey results from this biennium's work indicate that non-native tunicates are widespread throughout greater Puget Sound and it is likely that further surveys will uncover additional sites. High levels of infestation were evident at each of the six quantitatively assessed dock facilities and five were determined to be heavily infested with at least one of the three high priority species (*S. clava*, *D. vexillum*, *C. savignyi*) (Figure 30).

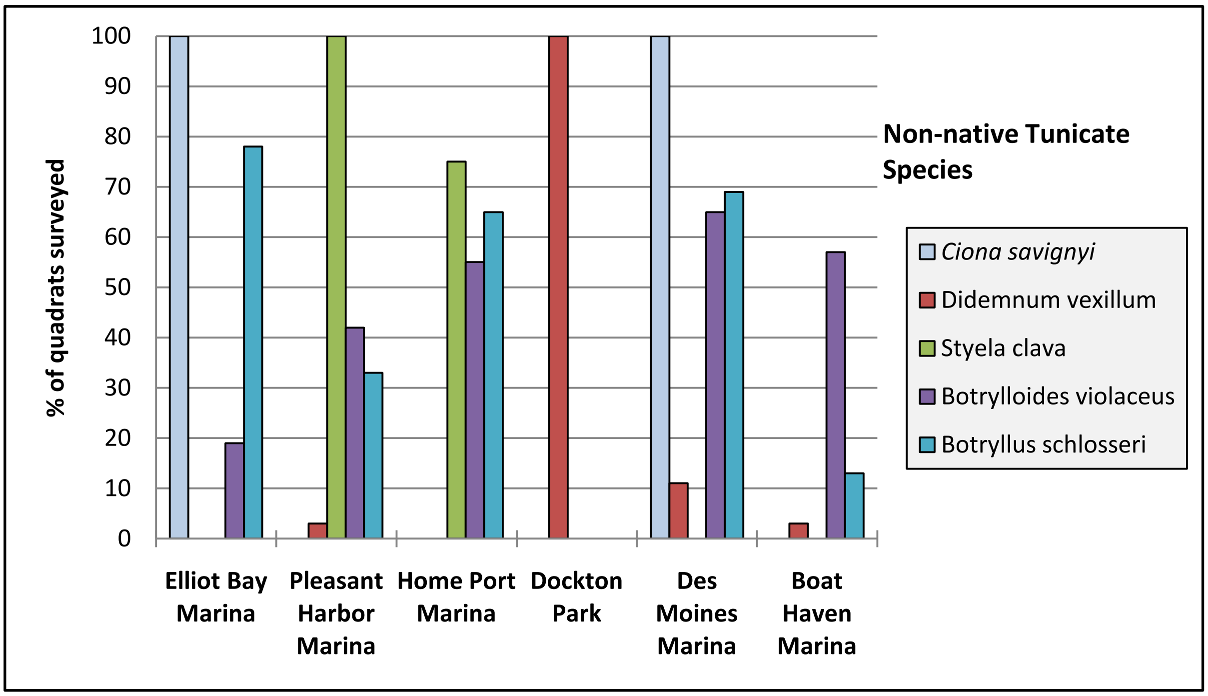


Figure 30. Distribution of five non-native tunicates among six quantitatively assessed dock facilities. The survey results presented for Dockton Park were derived from the pre-treatment assessment.

Small scale eradication treatment trials uncovered some promising methodologies which may, with further refinement, prove to be operationally and economically feasible at larger scales.

Based, in part, on the information presented in this report, and on advice from the TRAC, the AISU recommends that the following tasks be considered for inclusion in the 2009 biennium work plan, and that each of the tasks be prioritized according to available funding, staff, and the collective interests of the TRAC.

1) Continued long-term systematic monitoring of up to 50 sites throughout greater Puget Sound. Sites should include, at a minimum, marinas that are already infested or are believed to be at high risk of infestation, marine protected areas and ecologically sensitive marine habitat, and

areas that are in close proximity to aquaculture grow-out facilities and wild-stock shellfish harvest areas. Survey sites should contain substrate suitable for non-native tunicate settlement.

2) Convene a panel of ascidian experts, the primary function of which should be to conduct a thorough assessment of the ecologic and economic risks of non-native tunicate incursion into greater Puget Sound, and secondarily, to assist the AISU in establishing research priorities and make recommendations for control and eradication methods.

3) Community structure and *S. clava* density data should be collected from Blaine and Semiahmoo marinas using the same sampling protocol as was used this biennium at the six other quantitatively surveyed dock facilities that are infested with *S. clava*, *C. savignyi*, or *Didemnum vexillum* (Pleasant Harbor, Home Port, Elliot Bay, Des Moines, Boat Haven, and Dockton). The density data should be subjected to a statistical power analysis to determine the minimum, and thus most cost effective, sampling level that will enable the detection of density changes over time. Annual density surveys should then be conducted at each of the facilities using the prescribed sampling effort. Community structure information should be gathered from other marinas as time and resources permit.

4) Continue to conduct vessel hull removals of non-native tunicates from infested marinas in order to stem their spread to non-infested areas.

5) Continue to conduct small scale experiments aimed at determining threshold limits for those treatments that have proven to be effective, and continue to develop and test new approaches toward non-native tunicate control and eradication.

6) Design and conduct an ROV deepwater habitat survey of Hood Canal to determine the geographic and bathymetric distribution of *C. savignyi*. When/if co-occurrences are detected with geoduck, design and conduct field experiments aimed at assessing the impact of *C. savignyi* on geoduck growth, survival, and distribution. The AISU should explore the potential for a cost sharing collaboration with the WDFW sub-tidal shellfish unit to conduct this work.

7) A genetics study of the three priority non-native tunicate species should be conducted in order to ascertain the relationships among Puget Sound populations and to determine if source populations outside Puget Sound can be identified. The AISU should explore potential cost saving collaborations with Canadian researchers.